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<b>(54) Title:</b> DNA ENCODING A HUMAN NEUROPEPTIDE Y/PEPTIDE YY/PANCREATIC POLYPEPTIDE RECEPTOR (Y4) AND USES THEREOF			
<b>(57) Abstract</b> <p>This invention provides an isolated nucleic acid molecule encoding a human Y4 receptor, an isolated protein which is a human Y4 receptor, vectors comprising an isolated nucleic acid molecule encoding a human Y4 receptor, mammalian cells comprising such vectors, antibodies directed to the human Y4 receptor, nucleic acid probes useful for detecting nucleic acid encoding human Y4 receptors, antisense oligonucleotides complementary to any sequences of a nucleic acid molecule which encodes a human Y4 receptor, pharmaceutical compounds related to human Y4 receptors, and nonhuman transgenic animals which express DNA encoding a normal or a mutant human Y4 receptor. This invention further provides methods for determining ligand binding, detecting expression, drug screening, and treatment involving the human Y4 receptor.</p>			

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DNA ENCODING A HUMAN NEUROPEPTIDE  
Y/PEPTIDE YY/PANCREATIC POLYPEPTIDE RECEPTOR (Y4)  
AND USES THEREOF

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Background of the Invention

Throughout this application, various publications are referenced in parenthesis by Author and year. Full citations for these references may be found at the end of the specification immediately preceding the claims. The disclosure of these publications is hereby incorporated by reference into this application to describe more fully the art to which this invention pertains.

15

Neuropeptides are small peptides originating from large precursor proteins synthesized by peptidergic neurons and endocrine/paracrine cells. They hold promise for treatment of neurological, psychiatric, and endocrine disorders (De Wied, 1990). Often the precursors contain multiple biologically active peptides. There is great diversity of neuropeptides in the brain caused by alternative splicing of primary gene transcripts and differential precursor processing. The neuropeptide receptors serve to discriminate between ligands and to activate the appropriate signals. Thus, it is expected that the receptors for neuropeptides consist of a large number of members.

Neuropeptide Y (NPY), a 36-amino acid peptide, is the most abundant neuropeptide to be identified in mammalian brain. NPY is an important regulator in both the central and peripheral nervous systems (Heilig et al., 1990) and influences a diverse range of physiological parameters, including effects on psychomotor activity, food intake, central endocrine secretion, and vasoactivity in the cardiovascular system. High concentrations of NPY are found in the sympathetic nerves supplying the coronary, cerebral, and renal vasculature and has contributed to

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vasoconstriction. NPY binding sites have been identified in a variety of tissues, including spleen (Lundberg et al., 1988), intestinal membranes, brain (Hinson et al., 1988), aortic smooth muscle (Mihara et al., 1989),  
5 kidney, testis, and placenta (Dumont et al., 1992). In addition, binding sites have been reported in a number of rat and human cell lines (eg. Y1 in SK-N-MC, MC-IXC, CHP-212, and PC12 cells; Y2 in SK-N-Be(2), CHP-234, and SMS-MSN) (Aakerlund et al., 1990; Grundemar et al., 1993).

10

NPY forms a family (called the pancreatic polypeptide family) together with pancreatic polypeptide (PP) and peptide YY (PYY) which all consist of 36 amino acids and have a common tertiary structure, the so-called PP-fold  
15 (Glover et al., 1985). Specific features of this family include a polyproline helix in residues 1 through 8, a  $\beta$ -turn in residues 9 through 14, an  $\alpha$ -helix in residues 15 through 30, an outward-projecting C-terminus in residues 30 through 36, and a carboxy terminal amide which appears  
20 to be critical for biological activity (Schwartz et al., 1990). The C-terminal amidated residue of these peptides is essential for biological activity (Wahlestedt et al., 1986). Studies with peptide fragments of NPY have indicated that multiple NPY receptor subtypes exist  
25 (Wahlestedt et al., 1986). Three major NPY receptor subtypes (Y1, Y2 and Y3) have been defined by pharmacological criteria, with a fourth "atypical" Y1 receptor that has been proposed to regulate feeding behavior. The only NPY receptor which has been cloned to  
30 date is the Y1 receptor gene, from mouse (Eva et al., 1992), rat (Eva et al., 1990), and human (Larhammar et al., 1992). One of the key pharmacological features which distinguish Y1 and Y2 is the fact that the Y1 receptor (and not the Y2 receptor) responds to an analog  
35 of NPY modified at residues 31 and 34 ([Leu31,Pro34]NPY), whereas the Y2 receptor (and not the Y1 receptor) has high affinity for the NPY peptide carboxyl-terminal

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fragment NPY-(13-36) (Wahlstedt et al., 1986; Fuhlendorff et al., 1990).

Receptor genes for the other two structurally related  
5 peptides, peptide YY (PYY) and pancreatic polypeptide  
(PP), also have not been cloned. Peptide YY occurs  
mainly in endocrine cells in the lower gastrointestinal  
tract (Bottcher et al., 1984). Receptors for PYY were  
first described in the rat small intestine (Laburthe et  
10 al., 1986). This receptor has been defined as PYY-  
preferring because it displays a 5-10 fold higher  
affinity for PYY than for NPY (Laburthe et al., 1986;  
Laburthe, 1990). Recently, a cell line, PKSV-PCT,  
derived from the proximal tubules of kidneys, has been  
15 described to express receptors for PYY (Voisin et al.,  
1993). Pancreatic polypeptide is predominantly located  
in endocrine cells of the pancreatic islets (Alumets et  
al., 1978). PP inhibits pancreatic exocrine secretion  
and gall bladder contraction (Schwartz, 1983).  
20 Interestingly, PP does not appear to be synthesized in or  
localized to the central nervous system (Di Maggio et  
al., 1985), but selective PP binding sites have been  
found in various brain areas, such as the area postrema  
and adjacent nuclei, regions permeable at the blood-brain  
25 barrier (Whitcomb et al., 1990). PP receptors have a  
much higher affinity for PP than for NPY or PYY (Inui et  
al., 1990). PP has been shown to bind with high affinity  
to binding sites on a pheochromocytoma cell line, PC12  
(Schwartz et al., 1987). The rank order of affinity for  
30 the pharmacologically defined receptors of NPY and  
related peptides are listed in Table 1.

Using an homology screening approach to clone novel NPY  
receptor genes, we describe here the isolation and  
35 characterization of a novel NPY/PYY/PP receptor clone  
which we have designated Y4. The Y4 receptor appears to  
have a unique pharmacological profile, relative to other

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NPY-related receptors, exhibiting highest affinity for pancreatic polypeptide itself. This receptor clone will enable us to further examine the possibility of receptor diversity and the existence of multiple subtypes within  
5 this family of receptors. These could then serve as invaluable tools for drug design for several pathophysiological conditions such as memory loss, depression, anxiety, epilepsy, pain, hypertension,  
10 locomotor problems, circadian rhythm disorders, eating/body weight disorders, sexual/reproductive disorders, nasal congestion, diarrhea, gastrointestinal and cardiovascular disorders.

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Summary of the Invention

This invention provides an isolated nucleic acid molecule encoding a Y4 receptor.

5

This invention also provides an isolated protein which is a Y4 receptor.

This invention provides a vector comprising an isolated  
10 nucleic acid molecule encoding a Y4 receptor.

This invention also provides vectors such as plasmids and baculovirus comprising a nucleic acid molecule encoding a Y4 receptor, adapted for expression in a bacterial  
15 cell, a yeast cell, an insect cell or a mammalian cell which additionally comprise the regulatory elements necessary for expression of the nucleic acid in the bacterial, yeast, insect or mammalian cells operatively linked to the nucleic acid encoding the Y4 receptor as to  
20 permit expression thereof.

This invention provides a mammalian cell comprising nucleic acid encoding a Y4 receptor.

25 This invention provides a method for determining whether a ligand can specifically bind to a Y4 receptor which comprises contacting cell transfected with and expressing nucleic acid encoding a Y4 receptor with the ligand under conditions permitting binding of ligands to such Y4  
30 receptor, and detecting the presence of any of the ligand bound to a Y4 receptor, thereby determining whether the ligand binds specifically to a Y4 receptor.

This invention also provides a method for determining  
35 whether a ligand is a Y4 receptor agonist which comprises contacting a cell transfected with and expressing nucleic acid encoding a Y4 receptor with the ligand under

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conditions permitting the activation of a Y4 receptor functional response from the cell, and detecting by means of a bioassay, such as a second messenger response, an increase in Y4 receptor activity, thereby determining  
5 whether the ligand is a Y4 receptor agonist.

This invention further provides a method for determining whether a ligand is a Y4 receptor antagonist which comprises contacting a cell transfected with and  
10 expressing nucleic acid encoding a Y4 receptor with the ligand under conditions permitting the activation of a functional Y4 receptor response, and detecting by means of a bioassay, such as a second messenger response, a decrease in Y4 receptor activity, and thereby determining  
15 whether the ligand is a Y4 receptor antagonist.

This invention further provides a method of screening drugs to identify drugs which specifically bind to a Y4 receptor which comprises contacting a cell transfected  
20 with and expressing nucleic acid encoding a Y4 receptor with a plurality of drugs, and determining those drugs which bind to the cell, thereby identifying drugs which specifically bind to a Y4 receptor.

25 This invention also provides a method of screening drugs to identify which act as agonists of a Y4 receptor which comprises contacting a cell transfected with and expressing a Y4 receptor with a plurality of drugs under conditions permitting the activation of a functional Y4  
30 receptor response, and determining those drugs which activate the receptor in the cell using a bioassay such as a second messenger assay, thereby identifying drugs Y4 receptor agonists.

35 This invention also provides a method of screening drugs to identify drugs which act as antagonists of a Y4 receptor which comprises contacting a cell transfected



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with and expressing a Y4 receptor with a plurality of drugs in the presence of a known human Y4 receptor agonist, such as PP, under conditions permitting the activation of a functional Y4 receptor response and  
5 determining those drugs which inhibit the activation of the receptor in the cell using a bioassay, such as a second messenger assay, thereby identifying drugs which act as antagonists of a Y4 receptor.

10 This invention provides a nucleic acid probe comprising a nucleic acid molecule of at least 15 nucleotides capable of specifically hybridizing with a unique sequence included within the sequence of a nucleic acid molecule encoding a Y4 receptor.

15 This invention also provides a method of detecting expression of the Y4 receptor on the surface of a cell by detecting the presence of mRNA coding for a Y4 receptor which comprises obtaining total mRNA from the cell and  
20 contacting the mRNA so obtained with a nucleic acid probe comprising a nucleic acid molecule of at least 15 nucleotides capable of specifically hybridizing with a unique sequence included within the sequence of a nucleic acid molecule encoding a Y4 receptor under hybridizing  
25 conditions, detecting the presence of mRNA hybridized to the probe, and thereby detecting the expression of the Y4 receptor by the cell.

This invention provides an antisense oligonucleotide  
30 having a sequence capable of hybridizing specifically an mRNA molecule which encodes a Y4 receptor so as to prevent translation of the mRNA molecule.

This invention provides an antibody directed to a Y4  
35 receptor.

This invention provides a transgenic nonhuman mammal

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expressing nucleic acid encoding a Y4 receptor. This invention further provides a transgenic nonhuman mammal whose genome comprises antisense DNA complementary to DNA encoding a Y4 receptor so placed as to be transcribed  
5 into antisense mRNA which is complementary to mRNA encoding a Y4 receptor and which hybridizes to mRNA encoding a Y4 receptor thereby reducing its translation.

This invention provides a method of determining the  
10 physiological effects of expressing varying levels of Y4 receptors which comprises producing a transgenic nonhuman animal whose levels of Y4 receptor expression are varied by use of an inducible promoter which regulates Y4 receptor expression.

15 This invention also provides a method of determining the physiological effects of expressing varying levels of Y4 receptors which comprises producing a panel of transgenic nonhuman animals each expressing a different amount of Y4  
20 receptor.

This invention provides a method for diagnosing a predisposition to a disorder associated with the activity of a specific human Y4 receptor allele which comprises:  
25 a. obtaining nucleic acid of subjects suffering from the disorder; b. performing a restriction digest of the nucleic acid with a panel of restriction enzymes; c. electrophoretically separating the resulting nucleic acid fragments on a sizing gel; d. contacting the resulting  
30 gel with a nucleic acid probe capable of specifically hybridizing to nucleic acid encoding a Y4 receptor and labelled with a detectable marker; e. detecting labelled bands which have hybridized to the nucleic acid encoding a Y4 receptor labelled with a detectable marker to  
35 create a unique band pattern specific to the DNA of subjects suffering from the disorder; f. preparing nucleic acid obtained for diagnosis by steps a-e; and g.

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comparing the unique band pattern specific to the nucleic acid of subjects suffering from the disorder from step e and the nucleic acid obtained for diagnosis from step f to determine whether the patterns are the same or  
5 different and to diagnose thereby predisposition to the disorder if the patterns are the same.

This invention provides a method of preparing the purified, isolated Y4 receptor which comprises a)  
10 constructing a vector adapted for expression in a cell which comprises the regulatory elements necessary for the expression of nucleic acid in the cell operatively linked to the nucleic acid encoding a Y4 receptor as to permit expression thereof, wherein the cell is selected from the  
15 group consisting of bacterial cells, yeast cells, insect cells and mammalian cells; b) inserting the vector of step a in a suitable host cell; c) incubating the cells of step b under conditions allowing the expression of a Y4 receptor; d) recovering the receptor so produced; and e)  
20 purifying the receptor so recovered, thereby preparing an isolated, purified Y4 receptor.

This invention also provides a method of preparing the isolated Y4 receptor which comprises inserting nucleic  
25 acid encoding Y4 receptor in a suitable vector, inserting the resulting vector in a suitable host cell, recovering the receptor produced by the resulting cell, and purifying the receptor so recovered.

30 This invention provides an antisense oligonucleotide having a sequence capable of binding specifically with any sequences of an mRNA molecule which encodes the Y4 receptor so as to prevent translation of mRNA molecules which encode the Y4 receptor.

35 This invention also provides a transgenic nonhuman mammal expressing DNA encoding a human Y4 receptor.

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This invention further provides a transgenic nonhuman mammal comprising a homologous recombination knockout of the native Y4 receptor.

5 This invention also provides a method of determining the physiological effects of expressing varying levels of a receptor which comprises producing a transgenic nonhuman animal whose levels of human Y4 receptor expression are varied by use of an inducible promoter which regulates  
10 receptor expression.

This invention also provides a method of determining the physiological effects of expressing varying levels of a human Y4 receptor which comprises producing a panel of  
15 transgenic nonhuman animals each expressing a different amount of the receptor.

This invention further provides a transgenic nonhuman mammal whose genome comprises antisense DNA complementary  
20 to DNA encoding a human Y4 receptor so placed as to be transcribed into antisense mRNA which is complementary to mRNA encoding the receptor and which hybridizes to mRNA encoding the receptor thereby preventing its translation.

25 This invention provides a method for determining whether a ligand not known to be capable of binding to the Y4 receptor can bind to the receptor which comprises contacting a mammalian cell comprising an isolated DNA  
30 molecule encoding the Y4 receptor with the ligand under conditions permitting binding of ligands known to bind to the receptor, detecting the presence of any of the ligand bound to the Y4 receptor, and thereby determining whether the ligand binds to the Y4 receptor.

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Brief Description of the FiguresFigure 1

5 Nucleotide Sequence and Deduced Amino Acid Sequence of a  
 Novel Human hp25a Neuropeptide Receptor (Sequence I.D.  
 Nos. 1 and 2). Nucleotides are presented in the 5' to 3'  
 orientation and the coding region is numbered starting  
 from the initiating methionine and ending in the  
 10 termination codon. Deduced amino acid sequence by  
 translation of a long open reading frame is shown, along  
 with the 5' and 3' untranslated regions. Numbers in the  
 left and right margins represent nucleotide (top line)  
 and amino acid (bottom line) numberings, starting with  
 15 the first position as the adenosine (A) and the  
 initiating methionine (M), respectively.

Figure 2

Sequence Alignment of the Human hp25a clone with human  
 20 Y1, rat Y1, and mouse Y1 receptor genes. The deduced  
 amino acid sequence of the human hp25a (Y4) receptor  
 (first line), from the starting methionine (M) to the  
 stop codon (\*), is aligned with the human Y1 receptor  
 clone (Larhammar et al., 1992), rat Y1 receptor clone  
 25 (Eva et al., 1990), and mouse Y1 receptor clone (Eva et  
 al., 1992). Hyphens represent added spaces necessary for  
 proper alignment. Gray shading indicates residues in  
 receptor clones which are identical to hp25a. Numbers  
 above amino acid sequences correspond to amino acid  
 30 positions of hp78a, starting with the initiating  
 methionine (M) and ending with the termination codon (\*),  
 and including spaces to account for proper alignment.  
 Solid bars above the sequence indicate the seven putative  
 transmembrane (TM) spanning regions (TM I - VII).

35

Figure 3. Nucleotide sequence and deduced amino acid  
 sequence of the rat Y4 receptor encoded by rs16b

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(Sequence I.D. Nos and ). Nucleotides are presented in the 5' to 3' orientation and the coding region is numbered starting from the putative initiating methionine and ending in the termination codon. Deduced amino acid sequence by translation of a long open reading frame is shown, along with 5' and 3' untranslated regions. The amino acid sequence is represented using single-letter abbreviations.

- 10 Figure 4. Alignment of rat and human Y4 receptors. Predicted amino acid sequences of the rat Y4 receptor (Y4rat) and human Y4 receptor (Y4hum) are shown; the sequences are 75% identical overall and 84% identical in the transmembrane domains. Single letter abbreviations  
15 for amino acids are shown. The seven putative transmembrane (TM) spanning regions (TM I - VII) are indicated by brackets above the sequence.

Figure 5

- 20 Equilibrium binding of  $^{125}\text{I}$ -PYY to membranes from COS-7 cells transiently expressing hp25a receptors. Membranes were incubated with  $^{125}\text{I}$ -PYY for the times indicated, in the presence or absence of 100 nM human PP. Specific binding,  $B$ , was plotted against time,  $t$ , to obtain the  
25 maximum number of equilibrium binding sites,  $B_t$ , and observed association rate,  $K_{\text{obs}}$ , according to the equation,  $B = B_t * (1 - e^{-(K_{\text{obs}} * t)})$ . Binding is shown as the percentage of total equilibrium binding,  $B_t$ , determined by nonlinear regression analysis. Data are representative  
30 of three independent experiments, with each point measured in triplicate.

Figure 6A

- 35 Saturable equilibrium binding of  $^{125}\text{I}$ -PYY to membranes from COS-7 cells transiently expressing hp25a receptors. Membranes were incubated with  $^{125}\text{I}$ -PYY ranging in concentration from 0.003 nM to 2 nM, in the presence or

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absence of 100 nM human PP.

Figure 6B

Specific binding of the  $^{125}\text{I}$ -PYY to membranes from COS-7  
5 cells transiently expressing hp25a receptors under the  
conditions described in Figure 6A was plotted against the  
free  $^{125}\text{I}$ -PYY concentration,  $[\text{L}]$ , to obtain the maximum  
number of saturable binding sites,  $B_{\text{max}}$ , and the  $^{125}\text{I}$ -PYY  
equilibrium dissociation constant,  $K_d$ , according to the  
10 binding isotherm,  $B = B_{\text{max}}[\text{L}] / ([\text{L}] + K_d)$ . Specific binding  
is shown for data from a representative of four  
independent experiments, with each point measured in  
quadruplicate.

15 Figure 7. Competitive displacement of  $^{125}\text{I}$ -PYY from COS-7  
cells transiently expressing hp25a receptors. Membranes  
were incubated with  $^{125}\text{I}$ -PYY and increasing concentrations  
of peptide competitors.  $\text{IC}_{50}$  values corresponding to 50%  
displacement were determined by nonlinear regression  
20 analysis and converted to  $K_i$  values according to the  
equation,  $K_i = \text{IC}_{50} / (1 + [\text{L}] / K_d)$ , where  $[\text{L}]$  is the  $^{125}\text{I}$ -PYY  
concentration and  $K_d$  is the equilibrium dissociation  
constant of  $^{125}\text{I}$ -PYY. Data are representative of at least  
two independent experiments, with each point measured  
25 once or in duplicate. Rank orders of affinity for these  
and other compounds are listed separately in Table 2.

Figure 8. Inhibition of forskolin-stimulated cAMP  
30 accumulation in intact LM(tk-) cells stably expressing  
the human Y4 receptor. Functional data were derived  
from radioimmunoassay of cAMP in LM(tk-) cells stimulated  
with 10  $\mu\text{M}$  forskolin over a 5 minute period. Human PP was  
tested for agonist activity at concentrations ranging  
35 from 0.03 pM to 0.3  $\mu\text{M}$  over the same period. Data were  
fit to a four parameter logistic equation by nonlinear  
regression. The data shown are representative of three

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independent experiments.

Figures 9A and 9B. Figure 9A. Stimulation of intracellular free calcium concentration in intact LM(tk-  
5 ) cells stably expressing the human Y4 receptor. Representative time course. Functional data were derived from Fura-2/AM fluorescence in LM(tk-) cells stimulated with 100 nM human PP (open squares) or 100 nM human NPY (closed squares) at the time indicated by the arrow. The  
10 data shown are representative of two independent experiments. Figure 9B. Concentration/response curve. Data were fit to a four parameter logistic equation by nonlinear regression."



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Detailed Description of the Invention

Throughout this application, the following standard  
5 abbreviations are used to indicate specific nucleotide  
bases:

C = cytosine                      A = adenine

T = thymine                      G = guanine

This invention provides isolated nucleic acid molecules  
10 which encode Y4 receptors. In one embodiment the Y4  
receptor encoded is a rat Y4 receptor. In another  
embodiment, the Y4 receptor encoded is a human Y4  
receptor. In an embodiment, the isolated nucleic acid  
15 molecule encodes a Y4 receptor being characterized by an  
amino acid sequence in the transmembrane region, wherein  
the amino acid sequence has 60% homology or higher to the  
amino acid sequence in the transmembrane region of the  
human Y4 receptor shown in Figure 2. In another  
20 embodiment, the Y4 receptor has substantially the same  
amino acid sequence as the human Y4 receptor as described  
in Figure 1. In yet another embodiment, the Y4 receptor  
has substantially the same amino acid sequence as the rat  
Y4 receptor as described in Figure 3. In another  
25 embodiment, the Y4 receptor has the amino acid sequence  
as shown in Figure 1. In another embodiment, the Y4  
receptor has the amino acid sequence as shown in Figure  
3. As used herein, the term Y4 receptor encompasses any  
amino acid sequence, polypeptide or protein having  
substantially the same pharmacology provided subject  
30 human Y4 receptor as shown in Tables 1-3 and Table 6 and  
Figures 5-7. As described herein the human Y4 receptor  
has a pharmacological profile that differs from any known  
neuropeptide Y receptor subtype (i.e. Y1, Y2 and Y3),  
Neuropeptide YY receptor, and pancreatic polypeptide  
35 receptor, and is therefore designated as the human Y4  
receptor.

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The only NPY receptor which has been cloned to date is the Y1 receptor gene, from mouse (Eva et al., 1992), rat (Eva et al., 1990), and human (Larhammar et al., 1992). The Y4 receptor's greatest homology with any known  
5 receptor disclosed in the Genbank/EMBL databases is a 42% overall amino acid identity with the human Y1 receptor.

This invention provides an isolated nucleic acid molecule encoding a Y4 receptor. In one embodiment, the Y4  
10 receptor is a human Y4 receptor. In another embodiment, the Y4 receptor is a rat Y4 receptor. As used herein, the term "isolated nucleic acid molecule" means a nucleic acid molecule that is a molecule in a form which does not occur in nature. Examples of such an isolated nucleic  
15 acid molecule are an RNA, cDNA, or isolated genomic DNA molecule encoding a Y4 receptor. One means of isolating a human Y4 receptor is to probe a human genomic library with a natural or artificially designed DNA probe, using methods well known in the art. DNA probes derived from  
20 the human receptor gene Y4 are particularly useful probes for this purpose. DNA and cDNA molecules which encode human Y4 receptors may be used to obtain complementary genomic DNA, cDNA or RNA from human, mammalian or other animal sources, or to isolate related cDNA or genomic  
25 clones by the screening of cDNA or genomic libraries, by methods described in more detail below. Transcriptional regulatory elements from the 5' untranslated region of the isolated clones, and other stability, processing, transcription, translation, and tissue  
30 specificity-determining regions from the 3' and 5' untranslated regions of the isolated genes are thereby obtained. Examples of a nucleic acid molecule are an RNA, cDNA, or isolated genomic DNA molecule encoding a Y4 receptor. Such molecules may have coding sequences such  
35 as the coding sequences shown in Figures 1 or 3. The DNA molecule of Figure 1 encodes the amino acid sequence of a human Y4 receptor protein, while the DNA molecule of

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Figure 3 encodes the amino acid sequence of the rat Y4 receptor.

5 This invention further provides a cDNA molecule encoding a Y4 receptor having a coding sequence substantially the same as the coding sequence shown in Figures 1 and 3. This molecule is obtained by the means described above.

10 This invention also provides an isolated protein which is a Y4 receptor. In one embodiment, the Y4 receptor is a human Y4 receptor. In another embodiment, the Y4 receptor is a rat Y4 receptor. As used herein, the term "isolated protein" means a protein molecule free of other cellular components. An example of such a protein is an  
15 isolated protein having substantially the same amino acid sequence as the amino acid sequence shown in Figure 1 which is a human Y4 receptor or the amino acid sequence shown in Figure 3 which is a rat Y4 receptor. One means for obtaining isolated Y4 receptor is to express DNA  
20 encoding the receptor in a suitable host, such as a bacterial, yeast, insect or mammalian cell, using methods well known in the art, and recovering the receptor protein after it has been expressed in such a host, again using methods well known in the art. The receptor may  
25 also be isolated from cells which express it, in particular from cells which have been transfected with the expression vectors described below in more detail.

This invention provides vectors comprising isolated  
30 nucleic acid molecules such as DNA, RNA, or cDNA encoding a Y4 receptor. In one embodiment the Y4 receptor is a human Y4 receptor. In another embodiment the Y4 receptor is a rat Y4 receptor. Examples of vectors are viruses such as bacteriophages (such as phage lambda), animal  
35 viruses (such as Herpes virus, Murine Leukemia virus, and Baculovirus), cosmids, plasmids (such as pUC18, available from Pharmacia, Piscataway, NJ), and other recombination

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vectors. Nucleic acid molecules are inserted into vector genomes by methods well known in the art. For example, insert and vector DNA can both be exposed to a restriction enzyme to create complementary ends on both  
5 molecules which base pair with each other and are then ligated together with a ligase. Alternatively, linkers can be ligated to the insert DNA which correspond to a restriction site in the vector DNA, which is then  
10 digested with the restriction enzyme which cuts at that site. Other means are also available. Specific examples of such plasmids are plasmids comprising cDNA having a coding sequence substantially the same as the coding sequence shown in Figure 1 and designated clone hp25a (Seq. I.D. No. 1) or the coding sequence shown in Figure  
15 3 and designated clone rs16b (Sequence I.D. No. 27).

This invention also provides vectors comprising DNA molecules encoding Y4 receptors, adapted for expression in a bacterial cell, a yeast cell, an insect cell or a  
20 mammalian cell which additionally comprise the regulatory elements necessary for expression of the DNA in the bacterial, yeast, insect or mammalian cells operatively linked to the DNA encoding a Y4 receptor as to permit expression thereof. DNA having coding sequences  
25 substantially the same as the coding sequence shown in Figure 1 may usefully be inserted into the vectors to express human Y4 receptors. DNA having coding sequences substantially the same as the coding sequence shown in Figure 3 may usefully be inserted into the vectors to  
30 express rat Y4 receptors. Regulatory elements required for expression include promoter sequences to bind RNA polymerase and transcription initiation sequences for ribosome binding. For example, a bacterial expression vector includes a promoter such as the lac promoter and  
35 for transcription initiation the Shine-Dalgarno sequence and the start codon AUG (Maniatis, et al., Molecular Cloning, Cold Spring Harbor Laboratory, 1982).

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Similarly, a eukaryotic expression vector includes a heterologous or homologous promoter for RNA polymerase II, a downstream polyadenylation signal, the start codon AUG, and a termination codon for detachment of the ribosome. Furthermore, an insect expression vector, such as recombinant Baculovirus, uses the polyhedrin gene expression signals for expression of the inserted gene in insect cells. Such vectors may be obtained commercially or assembled from the sequences described by methods well known in the art, for example the methods described above for constructing vectors in general. Expression vectors are useful to produce cells that express the receptor. Certain uses for such cells are described in more detail below.

15 This invention further provides a plasmid adapted for expression in a bacterial, yeast, insect, or, in particular, a mammalian cell which comprises a DNA molecule encoding a Y4 receptor and the regulatory elements necessary for expression of the DNA in the bacterial, yeast, insect, or mammalian cell operatively linked to the DNA encoding a Y4 receptor as to permit expression thereof. Some plasmids adapted for expression in a mammalian cell are pSVL (available from Pharmacia, Piscataway, NJ) and pcEXV-3 (Miller J. and Germain R.N., J. Exp. Med. 164:1478 (1986)). A specific example of such plasmid is a plasmid adapted for expression in a mammalian cell comprising cDNA having coding sequences substantially the same as the coding sequence shown in Figure 1 and the regulatory elements necessary for expression of the DNA in the mammalian cell which is designated pcEXV-Y4 and deposited under ATCC Accession No. 75631. Another example of such plasmid is a plasmid adapted for expression in a mammalian cell comprising cDNA having coding sequences substantially the same as the coding sequence shown in Figure 3 and the regulatory elements necessary for expression of the DNA in the

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mammalian cell which is designated pcEXV-rY4 and deposited under ATCC Accession number \_\_\_\_\_. Those skilled in the art will readily appreciate that numerous plasmids adapted for expression in a mammalian cell which  
5 comprise DNA encoding Y4 receptors and the regulatory elements necessary to express such DNA in the mammalian cell may be constructed utilizing existing plasmids and adapted as appropriate to contain the regulatory elements necessary to express the DNA in the mammalian cell. The  
10 plasmids may be constructed by the methods described above for expression vectors and vectors in general, and by other methods well known in the art.

The deposit discussed supra, and the other deposits  
15 discussed herein, were made pursuant to, and in satisfaction of, the Budapest Treaty on the International Recognition of the Deposit of Microorganisms for the Purpose of Patent Procedure with the American Type Culture Collection (ATCC), 12301 Parklawn Drive,  
20 Rockville, Maryland 20852.

This invention provides a cell comprising a nucleic acid encoding a Y4 receptor, such as a mammalian cell comprising a plasmid adapted for expression in a  
25 mammalian cell, which comprises a nucleic acid molecule encoding a Y4 receptor, the protein encoded thereby is expressed on the cell surface, and the regulatory elements necessary for expression of the nucleic acid in the mammalian cell operatively linked to the nucleic acid  
30 encoding a Y4 receptor as to permit expression thereof. Numerous mammalian cells may be used as hosts, including, for example, the mouse fibroblast cell NIH-3T3, CHO cells, HeLa cells, LM(tk-) cells, Y1 cells, etc. Expression plasmids such as that described supra may be  
35 used to transfect mammalian cells by methods well known in the art such as calcium phosphate precipitation, or DNA encoding these Y4 receptors may be otherwise

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introduced into mammalian cells, e.g., by microinjection, to obtain mammalian cells which comprise DNA, e.g., cDNA or a plasmid, encoding either Y4 receptor. In one embodiment, the LM(tk-) cell is designated L-hY4-3 (ATCC  
5 Accession No.       ). In another embodiment, the NIH-3t3 cell is designated N-hY4-5 (ATCC Accession No.       ).

This invention provides a method for determining whether a ligand can specifically bind to a Y4 receptor which  
10 comprises contacting a cell transfected with and expressing nucleic acid encoding the Y4 receptor with the ligand under conditions permitting binding of ligands to such receptor, and detecting the presence of any such  
15 ligand bound specifically to the Y4 receptor, thereby determining whether the ligand binds specifically to a Y4 receptor. In one embodiment, the Y4 receptor is a human Y4 receptor. In another embodiment, the Y4 receptor is a rat Y4 receptor.

20 This invention provides a method for determining whether a ligand can specifically bind to a Y4 receptor which comprises contacting a cell transfected with and  
expressing nucleic acid encoding the Y4 receptor with the ligand under conditions permitting binding of ligands to  
25 such receptor, and detecting the presence of any such ligand bound specifically to the Y4 receptor, thereby determining whether the ligand binds specifically to a Y4 receptor, wherein the Y4 receptor is characterized by an amino acid sequence in the transmembrane region, wherein  
30 the amino acid sequence has 60% homology or higher to the amino acid sequence in the transmembrane region of the human Y4 receptor shown in Figure 2. In one embodiment, the Y4 receptor is a human Y4 receptor. In another embodiment, the Y4 receptor is a rat Y4 receptor.

35

This invention provides a method for determining whether a ligand can bind specifically to a Y4 receptor which

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comprises preparing a cell extract from cells transfected with and expressing nucleic acid encoding a Y4 receptor, isolating a membrane fraction from the cell extract, contacting the ligand with the membrane fraction under  
5 conditions permitting binding of ligands to such receptor, and detecting the presence of any ligand bound to the Y4 receptor, thereby determining whether the compound is capable of specifically binding to a Y4  
10 Y4 receptor. In one embodiment, the Y4 receptor is a human Y4 receptor. In another embodiment, the Y4 receptor is a rat Y4 receptor.

This invention provides a method for determining whether a ligand is a Y4 receptor agonist which comprises  
15 contacting a cell transfected with and expressing nucleic acid encoding a Y4 receptor with the ligand under conditions permitting the activation of a functional Y4 receptor response from the cell, and detecting by means of a bioassay, such as a second messenger response, an  
20 increase in Y4 receptor activity, thereby determining whether the ligand is a Y4 receptor agonist. In one embodiment, the Y4 receptor is a human Y4 receptor. In another embodiment, the Y4 receptor is a rat Y4 receptor.

25 This invention provides a method for determining whether a ligand is a Y4 receptor agonist which comprises preparing a cell extract from cells transfected with and expressing nucleic acid encoding a Y4 receptor, isolating a membrane fraction from the cell extract, contacting the  
30 membrane fraction with the ligand under conditions permitting the activation of a functional Y4 receptor response, and detecting by means of a bioassay, such as a second messenger response, an increase in Y4 receptor activity, thereby determining whether the ligand is a Y4  
35 receptor agonist. In one embodiment, the Y4 receptor is a human Y4 receptor. In another embodiment, the Y4 receptor is a rat Y4 receptor.



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This invention provides a method for determining whether a ligand is a Y4 receptor antagonist which comprises contacting a cell transfected with and expressing nucleic acid encoding a Y4 receptor with the ligand in the presence of a known Y4 receptor agonist, such as PP, under conditions permitting the activation of a functional Y4 receptor response and detecting by means of a bioassay, such as a second messenger response, a decrease in Y4 receptor activity, thereby determining whether the ligand is a Y4 receptor antagonist. In one embodiment, the Y4 receptor is a human Y4 receptor. In another embodiment, the Y4 receptor is a rat Y4 receptor.

This invention provides a method for determining whether a ligand is a Y4 receptor antagonist which comprises preparing a cell extract from cells transfected with and expressing nucleic acid encoding a Y4 receptor, isolating a membrane fraction from the cell extract, contacting the membrane fraction with the ligand in the presence of a known Y4 receptor agonist, such as PP, under conditions permitting the activation of a functional Y4 receptor response and detecting by means of a bioassay, such as a second messenger response, a decrease in Y4 receptor activity, thereby determining whether the ligand is a Y4 receptor antagonist. In one embodiment, the Y4 receptor is a human Y4 receptor. In another embodiment, the Y4 receptor is a rat Y4 receptor.

In one embodiment of the above-described methods, the ligand is not previously known.

This invention provides a Y4 receptor agonist detected by the above-described method. This invention provides a Y4 receptor antagonist detected by the above-described method.

As used herein, the term "agonist" means any ligand

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capable of increasing Y4 receptor activity. As used herein, the term "antagonist" means any ligand capable of decreasing Y4 receptor activity.

5 In one embodiment of the above-described methods, the cell is a mammalian cell. In a further embodiment, the cell is non-neuronal in origin. In another embodiment, the nonneuronal cell is a COS-7 cell, a CHO cell, an NIH-3T3 cell or an LM(tk-) cell.

10

One method for determining whether a ligand is capable of binding to the human Y4 receptor comprises contacting a transfected nonneuronal cell (i.e. a cell that does not naturally express any type of NPY, PP, or PYY receptor, thus will only express such a receptor if it is transfected into the cell) expressing a Y4 receptor on its surface, or contacting a membrane preparation derived from such a transfected cell, with the ligand under conditions which are known to prevail, and thus to be associated with, in vivo binding of the ligands to a Y4 receptor, detecting the presence of any of the ligand being tested bound to the Y4 receptor on the surface of the cell, and thereby determining whether the ligand binds to, activates or inhibits the activation of the Y4 receptor. A response system for detecting the activation or inhibition of activation of the Y4 receptor is obtained by transfection of isolated DNA into a suitable host cell containing the desired second messenger system such as phosphoinositide hydrolysis, adenylate cyclase, guanylate cyclase or ion channels. Such a suitable host cell system is isolated from pre-existing cell lines, or can be generated by inserting appropriate components of second messenger systems into existing cell lines. Such a transfection system provides a complete response system for investigation or assay of the activity of Y4 receptors with ligands as described above. Transfection systems are useful as living cell cultures for

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competitive binding assays between known or candidate drugs and ligands which bind to the receptor and which are labeled by radioactive, spectroscopic or other reagents. Membrane preparations containing the receptor  
5 isolated from transfected cells are also useful for these competitive binding assays. Functional assays of second messenger systems or their sequelae in transfection systems act as assays for binding affinity and efficacy in the activation of receptor function. A transfection  
10 system constitutes a "drug discovery system" useful for the identification of natural or synthetic compounds with potential for drug development that can be further modified or used directly as therapeutic compounds to activate or inhibit the natural functions of the Y4  
15 receptor. The transfection system is also useful for determining the affinity and efficacy of known drugs at the Y4 receptor sites.

This invention also provides a method of screening drugs  
20 to identify drugs which specifically bind to a Y4 receptor on the surface of a cell which comprises contacting a cell transfected with and expressing nucleic acid encoding a Y4 receptor with a plurality of drugs, and determining those drugs which bind to the cell,  
25 thereby identifying drugs which specifically bind to a Y4 receptor.

This invention also provides a method of screening drugs to identify drugs which specifically bind to a Y4  
30 receptor on the surface of a cell which comprises preparing a cell extract from cells transfected with and expressing nucleic acid encoding a Y4 receptor, isolating a membrane fraction from the cell extract, contacting the membrane fraction with a plurality of drugs, and  
35 determining those drugs which bind to the membrane fraction, thereby identifying drugs which specifically bind to a Y4 receptor.

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This invention also provides a method of screening drugs to identify drugs which act as Y4 receptor agonists which comprises contacting a cell transfected with and expressing nucleic acid encoding a Y4 receptor with a plurality of drugs under conditions permitting the activation of a functional Y4 receptor response, determining those drugs which activate the Y4 receptor in the cell using a bioassay, such as a second messenger assay, thereby identifying drugs which act as Y4 receptor agonists.

This invention also provides a method of screening drugs to identify drugs which act as Y4 receptor agonists which comprises preparing a cell extract from cells transfected with and expressing nucleic acid encoding a Y4 receptor, isolating a membrane fraction from the cell extract, contacting the membrane fraction with a plurality of drugs under conditions permitting the activation of a functional Y4 receptor response, determining those drugs which activate the Y4 receptor in the cell using a bioassay, such as a second messenger assay, thereby identifying drugs which act as Y4 receptor agonists.

This invention also provides a method of screening drugs to identify drugs which act as Y4 receptor antagonists which comprises contacting a cell transfected with and expressing DNA encoding a Y4 receptor with a plurality of drugs in the presence of a known Y4 receptor agonist, such as PP, under conditions permitting the activation of a functional Y4 receptor response, and determining those drugs which inhibit the activation of the Y4 receptor in the cell using a bioassay, such as a second messenger assay, thereby identifying drugs which act as Y4 receptor antagonists.

This invention also provides a method of screening drugs to identify drugs which act as Y4 receptor antagonists which comprises preparing a cell extract from cells

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transfected with and expressing nucleic acid encoding a Y4 receptor, isolating a membrane fraction from the cell extract, contacting the membrane fraction with a plurality of drugs in the presence of a known Y4 receptor agonist, such as PP, under conditions permitting the activation of a functional Y4 receptor response, and determining those drugs which inhibit the activation of the Y4 receptor in the cell using a bioassay, such as a second messenger assay, thereby identifying drugs which act as Y4 receptor antagonists.

In one embodiment of the above-identified methods, the Y4 receptor is a human Y4 receptor. In another embodiment, the Y4 receptor is a rat Y4 receptor. In one embodiment, the cell is a mammalian cell. In another embodiment, the mammalian cell is non-neuronal in origin. In a further embodiment, the mammalian cell non-neuronal in origin is a Cos-7 cell, a CHO cell, an LM(tk-) cell, a Y1 murine adrenal cell, or an NIH-3T3 cell.

The nucleic acid in the cell may have a coding sequence substantially the same as the coding sequences shown in Figures 1 and 3. Drug candidates are identified by choosing chemical compounds which bind with high affinity to the expressed Y4 receptor protein in transfected cells, using radioligand binding methods well known in the art, examples of which are shown in the binding assays described herein. Drug candidates are also screened for selectivity by identifying compounds which bind with high affinity to the Y4 receptor but do not bind with high affinity to any other NPY receptor subtype or to any other known receptor site. Because selective, high affinity compounds interact primarily with the target Y4 receptor site after administration to the patient, the chances of producing a drug with unwanted side effects are minimized by this approach.

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This invention provides a pharmaceutical composition comprising a drug identified by the method described above and a pharmaceutically acceptable carrier. As used herein, the term "pharmaceutically acceptable carrier" encompasses any of the standard pharmaceutical carriers, such as a phosphate buffered saline solution, water, and emulsions, such as an oil/water or water/oil emulsion, and various types of wetting agents. Once the candidate drug has been shown to be adequately bio-available following a particular route of administration, for example orally or by injection (adequate therapeutic concentrations must be maintained at the site of action for an adequate period to gain the desired therapeutic benefit), and has been shown to be non-toxic and therapeutically effective in appropriate disease models, the drug may be administered to patients by that route of administration determined to make the drug bio-available, in an appropriate solid or solution formulation, to gain the desired therapeutic benefit.

20

This invention provides a nucleic acid probe comprising a nucleic acid molecule of at least 15 nucleotides capable of specifically hybridizing with a unique sequence included within the coding sequence of a nucleic acid molecule encoding a Y4 receptor, for example with a coding sequence included within the sequences shown in Figures 1 and 3. In one embodiment, the nucleic acid encodes a human Y4 receptor. In another embodiment, the nucleic acid encodes a rat Y4 receptor. As used herein, the phrase "specifically hybridizing" means the ability of a nucleic acid molecule to recognize a nucleic acid sequence complementary to its own and to form double-helical segments through hydrogen bonding between complementary base pairs. As used herein, a "unique sequence" is a sequence specific to only the nucleic acid molecules encoding a Y4 receptor. Nucleic acid probe technology is well known to those skilled in the art who

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will readily appreciate that such probes may vary greatly in length and may be labeled with a detectable label, such as a radioisotope or fluorescent dye, to facilitate detection of the probe. Detection of nucleic acid encoding human Y4 receptors is useful as a diagnostic test for any disease process in which levels of expression of the corresponding Y4 receptor is altered. Nucleic acid probe molecules are produced by insertion of a nucleic acid molecule which encodes a Y4 receptor or fragments thereof into suitable vectors, such as plasmids or bacteriophages, followed by insertion into suitable bacterial host cells and replication and harvesting of the nucleic acid probes, all using methods well known in the art. For example, the nucleic acid may be extracted from a cell lysate using phenol and ethanol, digested with restriction enzymes corresponding to the insertion sites of the nucleic acid into the vector (discussed above), electrophoresed, and cut out of the resulting gel. Example of such nucleic acid molecules are shown in Figures 1 and 3. The probes are useful for 'in situ' hybridization or in order to locate tissues which express this gene family, or for other hybridization assays for the presence of these genes or their mRNA in various biological tissues. In addition, synthesized oligonucleotides (produced by a DNA synthesizer) complementary to the sequence of a DNA molecule which encodes a Y4 receptor or are useful as probes for these genes, for their associated mRNA, or for the isolation of related genes by homology screening of genomic or cDNA libraries, or by the use of amplification techniques such as the Polymerase Chain Reaction. Synthesized oligonucleotides as described may also be used to determine the cellular localization of the mRNA produced by the Y4 gene by in situ hybridization.

35

This invention also provides a method of detecting expression of a Y4 receptor by detecting the presence of

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mRNA coding for a Y4 receptor which comprises obtaining total mRNA from the cell using methods well known in the art and contacting the mRNA so obtained with a nucleic acid probe comprising a nucleic acid molecule of at least 5 15 nucleotides capable of specifically hybridizing with a sequence included within the sequence of a nucleic acid molecule encoding a Y4 receptor under hybridizing conditions, and detecting the presence of mRNA hybridized to the probe, thereby detecting the expression of the Y4 10 receptor by the cell. Hybridization of probes to target nucleic acid molecules such as mRNA molecules employs techniques well known in the art. In one possible means of performing this method, nucleic acids are extracted by precipitation from lysed cells and the mRNA is isolated 15 from the extract using a column which binds the poly-A tails of the mRNA molecules. The mRNA is then exposed to radioactively labelled probe on a nitrocellulose membrane, and the probe hybridizes to and thereby labels complementary mRNA sequences. Binding may be detected by 20 autoradiography or scintillation counting. However, other methods for performing these steps are well known to those skilled in the art, and the discussion above is merely an example.

25 This invention provides an antisense oligonucleotide having a sequence capable of specifically hybridizing with any sequences of an mRNA molecule which encodes a Y4 receptor so as to prevent translation of the mRNA molecule. The antisense oligonucleotide may have a 30 sequence capable of specifically hybridizing with any sequences of the cDNA molecule whose sequence is shown in Figure 1 or Figure 3. A particular example of an antisense oligonucleotide is an antisense oligonucleotide comprising chemical analogues of nucleotides.

35

This invention also provides a pharmaceutical composition comprising an amount of the oligonucleotide described



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above effective to reduce activity of a human Y4 receptor by passing through a cell membrane and specifically binding with mRNA encoding a Y4 receptor in the cell so as to prevent its translation and a pharmaceutically acceptable carrier capable of passing through a cell membrane. The oligonucleotide may be coupled to a substance which inactivates mRNA, such as a ribozyme. The pharmaceutically acceptable carrier capable of passing through cell membranes may also comprise a structure which binds to a receptor on a cell capable of being taken up by cells after binding to the structure. The structure of the pharmaceutically acceptable carrier may be capable of binding to a receptor which is specific for a selected cell type. The structure may be part of a protein known to bind a cell-type specific receptor, for example an insulin molecule, which would target pancreatic cells. Nucleic molecules having coding sequences substantially the same as the coding sequences shown in Figures 1 and 3 may be used as the oligonucleotides of the pharmaceutical composition.

This invention also provides a method of treating an abnormality wherein the abnormality is alleviated by decreasing the activity of a Y4 receptor which comprises administering to a subject an amount of the pharmaceutical composition described above effective to decrease the activity of the Y4 receptor. Several examples of such abnormal conditions are amnesia, anxiety, epilepsy, pain, hypertension, locomotor problems, circadian rhythm disorders, eating/body weight disorders, sexual/reproductive disorders, nasal congestion, diarrhea, gastrointestinal and cardiovascular disorders, and sleep and eating disorders.

Antisense oligonucleotide drugs inhibit translation of mRNA encoding these receptors. Synthetic oligonucleotides, or other antisense chemical structures

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are designed to bind to mRNA encoding the Y4 receptor and inhibit translation of mRNA and are useful as drugs to inhibit expression of Y4 receptor genes in patients. This invention provides a means to therapeutically alter

5 levels of expression of human Y4 receptors by the use of a synthetic antisense oligonucleotide drug (SAOD) which inhibits translation of mRNA encoding these receptors. Synthetic oligonucleotides, or other antisense chemical structures designed to recognize and selectively bind to

10 mRNA, are constructed to be complementary to portions of the nucleotide sequences shown in Figures 1 and 3 of DNA, RNA or of chemically modified, artificial nucleic acids. The SAOD is designed to be stable in the blood stream for administration to patients by injection, or in laboratory

15 cell culture conditions, for administration to cells removed from the patient. The SAOD is designed to be capable of passing through cell membranes in order to enter the cytoplasm of the cell by virtue of physical and chemical properties of the SAOD which render it capable

20 of passing through cell membranes (e.g. by designing small, hydrophobic SAOD chemical structures) or by virtue of specific transport systems in the cell which recognize and transport the SAOD into the cell. In addition, the SAOD can be designed for administration only to certain

25 selected cell populations by targeting the SAOD to be recognized by specific cellular uptake mechanisms which binds and takes up the SAOD only within certain selected cell populations. For example, the SAOD may be designed to bind to a receptor found only in a certain cell type,

30 as discussed above. The SAOD is also designed to recognize and selectively bind to the target mRNA sequence, which may correspond to a sequence contained within the sequences shown in Figures 1 and 3 by virtue of complementary base pairing to the mRNA. Finally, the

35 SAOD is designed to inactivate the target mRNA sequence by any of three mechanisms: 1) by binding to the target mRNA and thus inducing degradation of the mRNA by

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intrinsic cellular mechanisms such as RNase I digestion, 2) by inhibiting translation of the mRNA target by interfering with the binding of translation-regulating factors or of ribosomes, or 3) by inclusion of other  
5 chemical structures, such as ribozyme sequences or reactive chemical groups, which either degrade or chemically modify the target mRNA. Synthetic antisense oligonucleotide drugs have been shown to be capable of the properties described above when directed against mRNA  
10 targets (J.S. Cohen, Trends in Pharm. Sci. 10, 435 (1989); H.M. Weintraub, Sci. Am. January (1990) p. 40). In addition, coupling of ribozymes to antisense oligonucleotides is a promising strategy for inactivating target mRNA (N. Sarver et al., Science 247, 1222 (1990)).  
15 An SAOD serves as an effective therapeutic agent if it is designed to be administered to a patient by injection, or if the patient's target cells are removed, treated with the SAOD in the laboratory, and replaced in the patient. In this manner, an SAOD serves as a therapy to reduce  
20 receptor expression in particular target cells of a patient, in any clinical condition which may benefit from reduced expression of Y4 receptors.

This invention provides an antibody directed to a Y4  
25 receptor, for example a monoclonal antibody directed to an epitope of a Y4 receptor present on the surface of a cell and having an amino acid sequence substantially the same as an amino acid sequence for a cell surface epitope of the human Y4 receptor included in the amino acid  
30 sequence shown in Figure 1 (Seq. I.D. No. 2) or the rat Y4 receptor included in the amino acid sequence shown in Figure 3 (Seq. I.D. No. 28). Amino acid sequences may be analyzed by methods well known in the art to determine whether they produce hydrophobic or hydrophilic regions  
35 in the proteins which they build. In the case of cell membrane proteins, hydrophobic regions are well known to form the part of the protein that is inserted into the

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lipid bilayer which forms the cell membrane, while hydrophilic regions are located on the cell surface, in an aqueous environment. Therefore antibodies to the hydrophilic amino acid sequences shown in Figures 1 and 3 will probably bind to a surface epitope of a human or rat Y4 receptor, respectively, as described. Antibodies directed to Y4 receptors may be serum-derived or monoclonal and are prepared using methods well known in the art. For example, monoclonal antibodies are prepared using hybridoma technology by fusing antibody producing B cells from immunized animals with myeloma cells and selecting the resulting hybridoma cell line producing the desired antibody. Cells such as COS-7 cells or LM(tk-) cells comprising DNA encoding the human Y4 receptor and thereby expressing the human Y4 receptor may be used as immunogens to raise such an antibody. Alternatively, synthetic peptides may be prepared using commercially available machines and the amino acid sequences shown in Figures 1 and 3 (Seq. I.D. Nos. 2 and 28). As a still further alternative, DNA, such as a cDNA or a fragment thereof, may be cloned and expressed and the resulting polypeptide recovered and used as an immunogen. These antibodies are useful to detect the presence of human Y4 receptors encoded by the isolated DNA, or to inhibit the function of the receptors in living animals, in humans, or in biological tissues or fluids isolated from animals or humans.

This invention provides a pharmaceutical composition which comprises an amount of an antibody directed to the human Y4 receptor effective to block binding of ligands to the Y4 receptor, and a pharmaceutically acceptable carrier. A monoclonal antibody directed to an epitope of a Y4 receptor present on the surface of a cell and having an amino acid sequence substantially the same as an amino acid sequence for a cell surface epitope of the Y4 receptor included in the amino acid sequences shown in

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Figures 1 and 3 is useful for this purpose. In one embodiment, the Y4 receptor is a human Y4 receptor. In another embodiment, the Y4 receptor is a rat Y4 receptor.

5 This invention also provides a method of treating an abnormality wherein the abnormality is alleviated by decreasing the activity of a Y4 receptor which comprises administering to a subject an amount of the pharmaceutical composition described above effective to  
10 block binding of ligands to the Y4 receptor, thereby treating the abnormality. Binding of the antibody to the receptor prevents the receptor from functioning, thereby neutralizing the effects of Y4 receptor activity. The monoclonal antibodies described above are both useful for  
15 this purpose. Some examples of abnormalities are amnesia, depression, anxiety, epilepsy, pain, depression, hypertension, and sleep and eating disorders.

This invention provides a method of detecting the  
20 presence of a Y4 receptor on the surface of a cell which comprises contacting the cell with an antibody directed to the Y4 receptor, under conditions permitting binding of the antibody to the receptor, and detecting the presence of the antibody bound to the cell, thereby  
25 detecting the presence of the Y4 receptor on the surface of the cell. Such a method is useful for determining whether a given cell is defective in activity of Y4 receptors on the surface of the cell. Bound antibodies are detected by methods well known in the art, for  
30 example by binding fluorescent markers to the antibodies and examining the cell sample under a fluorescence microscope to detect fluorescence on a cell indicative of antibody binding. The monoclonal antibodies described above are useful for this purpose.

35

This invention provides a transgenic nonhuman mammal expressing nucleic acid encoding a Y4 receptor. This

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invention also provides a transgenic nonhuman mammal comprising a homologous recombination knockout of the native Y4 receptor. This invention also provides a transgenic nonhuman mammal whose genome comprises antisense DNA complementary to DNA encoding a Y4 receptor so placed as to be transcribed into antisense mRNA which is complementary to mRNA encoding a Y4 receptor and which hybridizes to mRNA encoding a Y4 receptor thereby reducing its translation. The DNA may additionally comprise an inducible promoter or additionally comprise tissue specific regulatory elements, so that expression can be induced, or restricted to specific cell types. Examples of DNA are DNA or cDNA molecules having a coding sequence substantially the same as the coding sequences shown in Figures 1 and 3. An example of a transgenic animal is a transgenic mouse. Examples of tissue specificity-determining regions are the metallothionein promoter (Low, M.J., Lechan, R.M., Hammer, R.E. et al. Science 231:1002-1004 (1986)) and the L7 promoter (Oberdick, J., Smeyne, R.J., Mann, J.R., Jackson, S. and Morgan, J.I. Science 248:223-226 (1990)).

Animal model systems which elucidate the physiological and behavioral roles of Y4 receptors are produced by creating transgenic animals in which the activity of a Y4 receptor is either increased or decreased, or the amino acid sequence of the expressed Y4 receptor protein is altered, by a variety of techniques. Examples of these techniques include: 1) Insertion of normal or mutant versions of DNA encoding a Y4 receptor or homologous animal versions of these genes, by microinjection, retroviral infection or other means well known to those skilled in the art, into appropriate fertilized embryos in order to produce a transgenic animal (Hogan B. et al. Manipulating the Mouse Embryo, A Laboratory Manual, Cold Spring Harbor Laboratory (1986)). 2) Homologous recombination (Capecchi M.R. Science 244:1288-1292

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(1989); Zimmer, A. and Gruss, P. Nature 338:150-153 (1989)) of mutant or normal, human or animal versions of these genes with the native gene locus in transgenic animals to alter the regulation of expression or the structure of these Y4 receptors. The technique of homologous recombination is well known in the art. It replaces the native gene with the inserted gene and so is useful for producing an animal that cannot express native receptor but does express, for example, an inserted mutant receptor, which has replaced the native receptor in the animal's genome by recombination, resulting in underexpression of the receptor. Microinjection adds genes to the genome, but does not remove them, and so is useful for producing an animal which expresses its own and added receptors, resulting in overexpression of the receptor. One means available for producing a transgenic animal, with a mouse as an example, is as follows: Female mice are mated, and the resulting fertilized eggs are dissected out of their oviducts. The eggs are stored in an appropriate medium such as M2 medium (Hogan B. et al. Manipulating the Mouse Embryo, A Laboratory Manual, Cold Spring Harbor Laboratory (1986)). DNA or cDNA encoding a human Y4 receptor is purified from a vector (such as plasmid pcEXV-Y4 described above) by methods well known in the art. Inducible promoters may be fused with the coding region of the DNA to provide an experimental means to regulate expression of the transgene. Alternatively or in addition, tissue specific regulatory elements may be fused with the coding region to permit tissue-specific expression of the transgene. The DNA, in an appropriately buffered solution, is put into a microinjection needle (which may be made from capillary tubing using a pipet puller) and the egg to be injected is put in a depression slide. The needle is inserted into the pronucleus of the egg, and the DNA solution is injected. The injected egg is then transferred into the oviduct of a pseudopregnant mouse (a

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mouse stimulated by the appropriate hormones to maintain pregnancy but which is not actually pregnant), where it proceeds to the uterus, implants, and develops to term.

As noted above, microinjection is not the only method  
5 for inserting DNA into the egg cell, and is used here only for exemplary purposes.

Since the normal action of receptor-specific drugs is to activate or to inhibit receptor activity, the transgenic  
10 animal model systems described above are useful for testing the biological activity of drugs directed against these Y4 receptors even before such drugs become available. These animal model systems are useful for predicting or evaluating possible therapeutic  
15 applications of drugs which activate or inhibit Y4 receptor activity by inducing or inhibiting expression of the native or trans-gene and thus increasing or decreasing activity of normal or mutant Y4 receptors in the living animal. Thus, a model system is produced in  
20 which the biological activity of drugs directed against these Y4 receptors are evaluated before such drugs become available. The transgenic animals which have increased or decreased Y4 receptor activity indicate by their physiological state whether increase or decrease of the  
25 Y4 receptor activity is therapeutically useful. It is therefore useful to evaluate drug action based on the transgenic model system. One use is based on the fact that it is well known in the art that a drug such as an antidepressant acts by blocking neurotransmitter uptake,  
30 and thereby increases the amount of neurotransmitter in the synaptic cleft. The physiological result of this action is to stimulate the production of less receptor by the affected cells, leading eventually to decreased activity of the receptor. Therefore, an animal which has  
35 decreased receptor activity is useful as a test system to investigate whether the actions of such drugs which result in decreased receptor activity are in fact



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therapeutic. Another use is that if increased receptor activity is found to lead to abnormalities, then a drug which down-regulates or acts as an antagonist to Y4 receptor is indicated as worth developing, and if a  
5 promising therapeutic application is uncovered by these animal model systems, activation or inhibition of Y4 receptor activity is achieved therapeutically either by producing agonist or antagonist drugs directed against these Y4 receptors or by any method which increases or  
10 decreases the activity of these Y4 receptors.

This invention provides a method of determining the physiological effects of expressing varying levels of human Y4 receptors which comprises producing a transgenic  
15 nonhuman animal whose levels of human Y4 receptor activity are varied by use of an inducible promoter which regulates Y4 receptor expression. This invention also provides a method of determining the physiological effects of expressing varying levels of Y4 receptors  
20 which comprises producing a panel of transgenic nonhuman animals each expressing a different amount of Y4 receptor activity. Such animals may be produced by introducing different amounts of nucleic acid encoding a Y4 receptor into the oocytes from which the transgenic animals are  
25 developed.

This invention also provides a method for identifying a Y4 receptor antagonist capable of alleviating an abnormality in a subject, wherein the abnormality is  
30 alleviated by decreasing the activity of a Y4 receptor which comprises administering the antagonist to a transgenic nonhuman mammal expressing at least one artificially introduced nucleic acid molecule encoding a Y4 receptor and determining whether the antagonist  
35 alleviates the physical and behavioral abnormalities displayed by the transgenic nonhuman mammal as a result of activity of a Y4 receptor, thereby identifying a Y4

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receptor antagonist. As used herein, the term "antagonist" means a compound or composition which may be natural, synthetic, or a product derived from screening. Examples of nucleic acid molecules are DNA, cDNA, genomic DNA, synthetic DNA or RNA molecules having coding sequences substantially the same as the coding sequences shown in Figures 1 and 3. This invention also provides an antagonist identified by the method described above.

- 10 This invention provides a pharmaceutical composition comprising an amount of the antagonist described supra and a pharmaceutically acceptable carrier.

15 This invention further provides a method for treating an abnormality in a subject wherein the abnormality is alleviated by decreasing the activity of a Y4 receptor which comprises administering to the subject an effective amount of the pharmaceutical composition described above, thereby treating the abnormality.

20 This invention provides a method for identifying a Y4 receptor agonist capable of alleviating an abnormality in a subject wherein the abnormality is alleviated by activation of a Y4 receptor which comprises administering the agonist to the transgenic nonhuman mammals described above determining whether the agonist alleviates the physical and behavioral abnormalities displayed by the transgenic nonhuman mammal, the alleviation of the abnormality indicating the identification of a Y4 receptor agonist.

This invention provides an agonist identified by the method described above.

- 35 This invention also provides a pharmaceutical composition comprising an amount of the agonist identified by the method described above and a pharmaceutically acceptable

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carrier.

This invention further provides a method for treating an abnormality in a subject wherein the abnormality is  
5 alleviated by activation of a Y4 receptor which comprises administering to a subject an effective amount of the pharmaceutical composition described above, thereby treating the abnormality.

10 This invention provides a method for diagnosing a predisposition to a disorder associated with the activity of a specific Y4 receptor allele which comprises: a) obtaining DNA of subjects suffering from the disorder; b) performing a restriction digest of the DNA with a panel  
15 of restriction enzymes; c. electrophoretically separating the resulting DNA fragments on a sizing gel; d) contacting the resulting gel with a nucleic acid probe capable of specifically hybridizing to DNA encoding a Y4 receptor and labelled with a detectable marker; e)  
20 detecting labelled bands which have hybridized to the DNA encoding a Y4 receptor labelled with a detectable marker to create a unique band pattern specific to the DNA of subjects suffering from the disorder; f) preparing DNA obtained for diagnosis by steps a-e; and g) comparing  
25 the unique band pattern specific to the DNA of subjects suffering from the disorder from step e and the DNA obtained for diagnosis from step f to determine whether the patterns are the same or different and thereby to diagnose predisposition to the disorder if the patterns  
30 are the same. This method may also be used to diagnose a disorder associated with the expression of a specific Y4 receptor allele.

This invention provides a method of preparing the  
35 purified, isolated Y4 receptor which comprises a) constructing a vector adapted for expression in a cell which comprises the regulatory elements necessary for the

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expression of nucleic acid in the cell operatively linked to the nucleic acid encoding a Y4 receptor as to permit expression thereof, wherein the cell is selected from the group consisting of bacterial cells, yeast cells, insect cells and mammalian cells; b) inserting the vector of step a in a suitable host cell; c) incubating the cells of step b under conditions allowing the expression of a Y4 receptor; d) recovering the receptor so produced; and e) purifying the receptor so recovered, thereby preparing the purified, isolated Y4 receptor. An example of an isolated Y4 receptor is an isolated protein having substantially the same amino acid sequence as the amino acid sequences shown in Figures 1 and 3. For example, cells can be induced to express receptors by exposure to substances such as hormones. The cells can then be homogenized and the receptor isolated from the homogenate using an affinity column comprising, for example, PP or another substance which is known to bind to the receptor. The resulting fractions can then be purified by contacting them with an ion exchange column, and determining which fraction contains receptor activity or binds anti-receptor antibodies. This method for preparing Y4 receptor uses recombinant DNA technology methods well known in the art. For example, isolated nucleic acid encoding Y4 receptor is inserted in a suitable vector, such as an expression vector. A suitable host cell, such as a bacterial cell, or a eukaryotic cell such as a yeast cell, is transfected with the vector. Y4 receptor is isolated from the culture medium by affinity purification or by chromatography or by other methods well known in the art.

This invention identifies for the first time a new receptor protein, its amino acid sequence, and its human gene. Furthermore, this invention describes a previously unrecognized group of receptors within the definition of a Y4 receptor. The information and experimental tools

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provided by this discovery are useful to generate new therapeutic agents, and new therapeutic or diagnostic assays for this new receptor protein, its associated mRNA molecule or its associated genomic DNA. The information  
5 and experimental tools provided by this discovery will be useful to generate new therapeutic agents, and new therapeutic or diagnostic assays for this new receptor protein, its associated mRNA molecule, or its associated genomic DNA.

10

Specifically, this invention relates to the first isolation of a human and a rat genomic clone encoding a Y4 receptor. A new human gene for the receptor identified herein as Y4 has been identified and  
15 characterized. In addition, the human Y4 receptor has been expressed in COS-7 cells. The pharmacological binding properties of the protein encoded have been determined, and these binding properties classify this protein as a novel NPY/PYY/PP receptor which we designate  
20 as a Y4 receptor. Mammalian cell lines expressing this Y4 receptor at the cell surface have been constructed, thus establishing the first well-defined, cultured cell lines with which to study this Y4 receptor.

25 This invention will be better understood by reference to the Experimental Details which follow, but those skilled in the art will readily appreciate that the specific experiments detailed are only illustrative of the invention as described more fully in the claims which  
30 follow thereafter.

Experimental Details

Cloning and Sequencing of a human (Y4) Neuropeptide  
5 Receptor. A human placenta genomic library in  $\lambda$  dash II  
( $\approx 1.5 \times 10^6$  total recombinants; Stratagene, LaJolla, CA)  
was screened using overlapping transmembrane (TM)  
oligonucleotide probes (TM 1, 2, 3, 5 and 7) derived from  
the rat Y1 neuropeptide receptor gene (Eva, C. et al.,  
10 1990; GenBank accession No. Z11504). Overlapping  
oligomers (TM1: nts. 198-251, (+)strand/5'-  
TTGCTTATGGGGCTGTGATTATTCTTGGGGTCTCTGGAAACCTGG-3'  
(Sequence I.D. No. 3) and (-)strand/5'-  
TAGGATGATTATGATCAATGCCAGGTTTCCAGAGACCCCAAGAAT-3'  
15 (Sequence I.D. No. 4); TM2: nts. 269-328, (+)strand/5'-  
AAAGAGATGAGGAATGTCACCAACATTCTGATCGT  
GAACCTCTCC-3' (Sequence I.D. No. 5) and (-)strand/5'-  
CAGCAAGTCTGAGAAGGAGAGGTTACGATCAGAATGTTGGTGAC-3'  
(Sequence I.D. No. 6); TM3: nts. 401-478, (+)strand/5'-  
20 TGCAAACTGAATCCTTTTGTGCAATGCGTCTCCATTACAGTATCCATTTTCTCT-3'  
(Sequence I.D. No. 7) and (-) strand/5'-ACGTTCCACAGC  
GATGAGAACCAGAGAGAAAATGGATACTGTAATGGAGACGCA-3' (Sequence  
I.D. No. 8); TM5: nts. 716-778, (+)strand/5'-  
CTGCAGTATTTTGGCCCACTCTGTTTCATATTCATATGCTAC-3' (Sequence  
25 I.D. No. 9) and (-)strand/5'-  
CAAGCGAATGTATATCTTGAAGTAGCATATGAATATGAAACA-3' (Sequence  
I.D. No. 10); TM7: nts. 971-1045, (+)strand/5'-  
CTGCTCTGCCACCTCACGGCCATGATCTCCACCTGCGTCAACC  
CCATC-3' (Sequence I.D. No. 11) and (-)strand/5'-  
30 GAAATTTTGTTCAGGAATCCATAAAAGATGGGGTTGA  
CGCAGGTGGA-3' (Sequence I.D. No. 12); GenBank accession  
No. Z11504) were labeled with [ $^{32}$ P]dATP and [ $^{32}$ P]dCTP by  
synthesis with the large fragment of DNA polymerase.  
Hybridization was performed at low stringency conditions:  
35 40°C. in a solution containing 25.0% formamide, 5x SSC  
(1x SSC is 0.15M sodium chloride, 0.015M sodium citrate),  
1x Denhardt's solution (0.02% polyvinylpyrrolidone,

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0.02% Ficoll, 0.02% bovine serum albumin), and 25  $\mu\text{g}/\mu\text{l}$  sonicated salmon sperm DNA. The filters were washed at 40°C. in 0.1x SSC containing 0.1% sodium dodecyl sulfate and exposed at -70°C. to Kodak XAR film in the presence  
5 of an intensifying screen. Lambda phage clones hybridizing with the probes were plaque purified and DNA was prepared for Southern blot analysis (Southern, 1975; Sambrook et al., 1989). A Genomic clone hybridizing with all five of the rat Y1 TM probes, designated hp25a,  
10 was isolated using this method. For subcloning and further Southern blot analysis, the hp25a DNA was cloned into pUC18 (Pharmacia, Piscataway, NJ). Nucleotide sequence analysis was accomplished by the Sanger dideoxy nucleotide chain termination method (Sanger et al., 1977)  
15 on denatured double-stranded plasmid templates, using Sequenase (US Biochemical Corp., Cleveland, OH).

Cloning and Sequencing of a rat NPY (Y4) neuropeptide receptor:

20 A rat spleen genomic library (Stratagene, La Jolla, CA) was screened using overlapping TM oligonucleotide probes (TM 1 - 7) derived from the nucleotide sequences corresponding approximately to the TM regions of the amino acid sequence of the human Y4 receptor as shown in  
25 Figure 2. The overlapping oligomers used were as follows:

TM1: nts. #129-201,

(+) strand/5'-TCATCGTCACTTCCTACAGCATTGAGACTGTCGTGG  
GGGTCCTGGGT (Sequence I.D. No. ) and

(-) strand/5'-ACAGTCACACACATCAGGCAGAGGTTACCCAGGAC  
30 CCCCACGACAG (Seq. I.D. No. );

TM2: nts. #234-303,

( + ) s t r a n d / 5 ' -  
TGCTTATCGCCAACCTGGCCTTCTCTGACTTCCTCATGTGCCTCC (Seq.  
I.D. No. ) and

35 ( - ) s t r a n d / 5 ' -  
TAGACGGCGGTCAGCGGCTGGCAGAGGAGGCACATGAGGAAGTCA (Seq.  
I.D. No. );

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TM3: nts. #348-417,  
( + ) s t r a n d / 5 ' -  
TGTCGGCCTTCATCCAGTGCATGTCGGTGACGGTCTCCATCCTCT (Seq.  
I.D. No. ) and  
5 ( - ) s t r a n d / 5 ' -  
CTCTCCAGGGCCACGAGGACGAGCGAGAGGATGGAGACCGTCACC (Seq.  
I.D. No. );  
TM4: nts. #467-536,  
( + ) s t r a n d / 5 ' -  
10 GCCTACCTGGGGATTGTGCTCATCTGGGTCATTGCCTGTGTCCTC (Seq.  
I.D. No. ) and  
( - ) s t r a n d / 5 ' -  
TGCTGTTGGCCAGGAAGGGCAGGGAGAGGACACAGGCAATGACCC (Seq.  
I.D. No. );  
15 TM5: nts. #637-706,  
( + ) s t r a n d / 5 ' -  
CATCTACACCACCTTCCTGCTCCTCTTCCAGTACTGCCTCCCACT (Seq.  
I.D. No. ) and  
( - ) s t r a n d / 5 ' -  
20 TGCATAACAGACCAGGATGAAGCCCAGTGGGAGGCAGTACTGGAA (Seq.  
I.D. No. );  
TM6: nts. #800-870,  
(+) strand/5'-CTGGTGGTGATGGTGGTGGCCTTTGCCGTGCTCT  
GGCTGCCTCTGC (Seq. I.D. No. ) and  
25 ( - ) s t r a n d / 5 ' -  
CAGTCTTCCAGGCTGTTGAACACATGCAGAGGCAGCCAGAGCACG (Seq.  
I.D. No. );  
TM7: NTS. #908-977,  
( + ) s t r a n d / 5 ' -  
30 ATCTTCTTAGTGTGCCACTTGCTTGCCATGGCCTCCACCTGCGTC (Seq.  
I.D. No. ) and  
( - ) s t r a n d / 5 ' -  
TGAGAAAGCCATAGATGAATGGGTTGACGCAGGTGGAGGCCATGG (Seq.  
I.D. No. ) were labeled with [<sup>32</sup>P]-ATP and [<sup>32</sup>P]-CTP  
35 by synthesis with the large fragment of DNA  
polymerase. Hybridization was performed at reduced  
stringency conditions: 40°C in a solution



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containing 37.5% formamide, 10% dextran sulfate, 5X SSC, 1X Denhardt's solution, and 100 µg/ml of sonicated salmon sperm DNA. The filters were washed at 45°C in 0.1X SSC containing 0.1% sodium dodecyl sulfate (SDS) and exposed at -70°C to Kodak XAR film in the presence of an intensifying screen. Lambda phage clones hybridizing to the probes were plaque purified by successive plating and rescreening. A genomic clone hybridizing with all seven human Y4 receptor TM probes, designated rs16b, was isolated using this method. For expression and sequence analysis, a 2.0 kb BamHI/HindIII fragment of rs16b was subcloned into the corresponding polylinker sites of a pcEXV-3 eukaryotic expression vector (Miller and Germain, 1986) modified to include a polylinker with EcoRI, SstI, ClaI, KpnI, SmaI, XbaI, BamHI, SalI and HindIII restriction sites and designated EXJ.RH. Nucleotide sequence analysis was accomplished by the Sanger dideoxy nucleotide chain-termination method (Sanger, 1977) on double stranded plasmid templates, using Sequenase (U.S. Biochemical Corp., Cleveland, Ohio).

Transient Transfection: The entire coding region of hp25a (1127 bp), including 680 bp of 5' untranslated (5' UT) and 205 bp of 3' untranslated sequence (3' UT), was cloned into the BamHI and EcoRI sites of the polylinker-modified eukaryotic expression vector pCEXV-3 (Miller et al., 1986), called EXJ.HR (J.B., unpublished data). Monkey kidney cells (Cos-7) were transiently transfected with plasmid hp25a/EXJ (expression vector containing the hp25a receptor gene) using DEAE dextran methodology (reagents obtained from Specialty Media, Lavellette, NJ).

The plasmid rs16b/EXJ (the expression vector containing

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the rs16b receptor gene), was transiently transfected into Cos-7 cells using similar methods, as were the human Y1 receptor (Larhammar, 1992) and the human Y2 receptor. The cloned Y2 receptor was disclosed in U.S. patent application 08/192,288 filed on February 2, 1994, currently pending, the foregoing contents of which are hereby incorporated by reference.

#### Stable Transfection

Human Y4 receptors were co-transfected with a G-418 resistant gene into the mouse embryonic NIH-3T3 cell line by a calcium phosphate transfection method (Cullen, 1987). Stably transfected cells were selected with G-418. Human Y4 receptors were similarly transfected into mouse fibroblast LM(tk-) cells.

Cell culture: COS-7 cells were grown on 150 mm plates (Corning) in D-MEM with supplements (Dulbecco's Modified Eagle Medium with 10% bovine calf serum, 2 mM glutamine, 100 units/ml penicillin/80 units/ml streptomycin) at 37 °C, 5% CO<sub>2</sub>. Stock plates of COS-7 cells were trypsinized and split 1:6 every 3-4 days. SK-N-Be(2) human neuroblastoma cells were grown similarly in 225 cm<sup>2</sup> flasks (Co-star) using 50% Eagle's Modified Essential Media, 50% Ham's Nutrient Mixture F-12, 15% fetal bovine serum, 2 mM glutamine, 100 units/ml penicillin/80 units/ml streptomycin, and 1% non-essential amino acids. Stock flasks of SK-N-Be(2) cells were trypsinized and split 1:10 every 7 days.

30

Mouse embryonic NIH-3T3 cells were grown on 150 mm plates in Dulbecco's Modified Eagle Medium (DMEM) with supplements (10% bovine calf serum, 4 mM glutamine, 100 units/ml penicillin/100 µg/ml streptomycin) at 37 °C, 5% CO<sub>2</sub>. Stock plates of NIH-3T3 cells were trypsinized and split 1:15 every 3-4 days. Mouse fibroblast LM(tk-)

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cells were grown on 150 mm plates in Dulbecco's Modified Eagle Medium (DMEM) with supplements (10% bovine calf serum, 4 mM glutamine, 100 units/ml penicillin/100 µg/ml streptomycin) at 37 °C, 5% CO<sub>2</sub>. Stock plates of LM(tk-)  
5 cells were trypsinized and split 1:10 every 3-4 days.

Cell culture media and supplements were from Specialty Media (Lavallette, NJ). Cell culture plates (150 mm) were from Corning (Corning, NY). Cell culture flasks (225 cm<sup>2</sup>)  
10 and polypropylene microtiter plates were from Co-star (Cambridge, MA).

Membrane Harvest: Membranes were harvested from COS-7 cells 48 hours after transfection and from SK-N-Be(2) seven days after splitting. Adherent cells were washed  
15 twice in ice-cold phosphate buffered saline (138 mM NaCl, 8.1 mM Na<sub>2</sub>HPO<sub>4</sub>, 2.5 mM KCl, 1.2 mM KH<sub>2</sub>PO<sub>4</sub>, 0.9 mM CaCl<sub>2</sub>, 0.5 mM MgCl<sub>2</sub>, pH 7.4) and lysed by sonication in ice-cold hypotonic buffer (20 mM Tris-HCl, 5 mM EDTA, pH 7.7).  
20 Large particles and debris were cleared by low speed centrifugation (200 x g, 10 min, 4 °C). Membranes were collected from the supernatant fraction by high speed centrifugation (32,000 x g, 18 min, 4 °C), washed with ice-cold hypotonic buffer, and collected again by high  
25 speed centrifugation (32,000 x g, 18 min, 4 °C). The final membrane pellet was resuspended by sonication into a small volume (~500 µl) of ice-cold binding buffer (10 mM NaCl, 20 mM HEPES, 0.22 mM KH<sub>2</sub>PO<sub>4</sub>, 1.26 mM CaCl<sub>2</sub>, 0.81 mM MgSO<sub>4</sub>, pH 7.4). Protein concentration was measured by the  
30 Bradford method (Bradford, 1976) using Bio-Rad Reagent, with bovine serum albumin as a standard.

Radioligand Binding to Membrane Suspensions: Membrane suspensions were diluted in binding buffer supplemented  
35 with 0.1% bovine serum albumin and 0.1% bacitracin to yield an optimal membrane protein concentration: ~ 0.02 mg/ml for human Y1 receptors, ~ 0.015 mg/ml for hp25a

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- receptors, and ~ 0.25 mg/ml for SK-N-Be(2). (Under these conditions,  $^{125}\text{I}$ -PYY bound by membranes in the assay was less than 10% of  $^{125}\text{I}$ -PYY delivered to the sample.)  $^{125}\text{I}$ -PYY and non-labeled peptide competitors were also diluted to
- 5 desired concentrations in supplemented binding buffer. Individual samples were then prepared in 96-well polypropylene microtiter plates by mixing membrane suspensions (200 ul),  $^{125}\text{I}$ -PYY (25 ul), and non-labeled peptides or supplemented binding buffer (25 ul).
- 10 Samples were incubated in a 30 °C water bath with constant shaking for 120 min. Incubations were terminated by filtration over Whatman GF/C filters (pre-coated with 0.5% polyethyleneimine and air-dried before use). Filter-trapped membranes were counted for  $^{125}\text{I}$  in a gamma counter.
- 15 Non-specific binding was defined by 100 nM human PP for hp25a receptors and by 100 nM NPY for Y1 and SK-N-Be(2) receptors. Specific binding in time course and competition studies was typically 80%; most non-specific binding was associated with the filter. Binding data were
- 20 analyzed using nonlinear regression and statistical techniques available in the GraphPAD InPlot package (San Diego, CA). Porcine  $^{125}\text{I}$ -PYY was from New England Nuclear (Boston, MA). NPY and related peptide analogs were from either Bachem
- 25 California (Torrance, CA) or Peninsula (Belmont, CA). Whatman GF/C filters were from Brandel (Gaithersburg, MD). Bio-Rad Reagent was from Bio-Rad (Hercules, CA). Bovine serum albumin and bacitracin were from Sigma (St. Louis, MO). All other materials were reagent grade.
- 30 Functional Assay: Radioimmunoassay of cAMP
- Stably transfected cells were seeded into 96-well microtiter plates and cultured until confluent. To reduce the potential for receptor desensitization, the serum component of the media was reduced to 1.5% for 4 to 16
- 35 hours before the assay. Cells were washed in Hank's buffered saline, or HBS (150 mM NaCl, 20 mM HEPES, 1 mM  $\text{CaCl}_2$ , 5 mM KCl, 1 mM  $\text{MgCl}_2$ , and 10 mM glucose)

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supplemented with 0.1% bovine serum albumin plus 5 mM theophylline and pre-equilibrated in the same solution for 20 min at 37 °C in 5% CO<sub>2</sub>. Cells were then incubated 5 min with 10 μM forskolin and various concentrations of  
5 receptor-selective ligands. The assay was terminated by the removal of HBS and acidification of the cells with 100 mM HCl. Intracellular cAMP was extracted and quantified with a modified version of a magnetic bead-based radioimmunoassay (Advanced Magnetics, Cambridge,  
10 MA). The final antigen/antibody complex was separated from free <sup>125</sup>I-cAMP by vacuum filtration through a PVDF filter in a microtiter plate (Millipore, Bedford, MA). Filters were punched and counted for <sup>125</sup>I in a Packard gamma counter. Binding data were analyzed using nonlinear  
15 regression and statistical techniques available in the GraphPAD Prism package (San Diego, CA).

#### Functional Assay: Intracellular Calcium Mobilization

The intracellular free calcium concentration was  
20 measured by microspectrofluorometry using the fluorescent indicator dye Fura-2/AM. Stably transfected cells were seeded onto a 35 mm culture dish containing a glass coverslip insert. Cells were washed with HBS and then loaded with 100 μl of Fura-2/AM (10 μM) for 20 to 40 min.  
25 After washing with HBS to remove the Fura-2/AM solution, cells were equilibrated in HBS for 10 to 20 min. Cells were then visualized under the 40X objective of a Leitz Fluovert FS microscope and fluorescence emission was determined at 510 nm with excitation wave lengths  
30 alternating between 340 and 380 nm. Raw fluorescence data were converted to calcium concentrations using standard calcium concentration curves and software analysis techniques.

35. Tissue Localization and Gene Expression: Reverse Transcriptase PCR  
Human tissues (obtained from National Disease Research

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Interchange) were homogenized and total RNA extracted using guanidine isothiocyanate/CsCl cushion method (Kingston, 1987). RNA was treated with DNase to remove any contaminating genomic DNA. cDNA was prepared from  
5 total RNA with random hexanucleotide primers using reverse transcriptase (Superscript II; BRL). An aliquot of the first strand cDNA (250ng of total RNA) was amplified in a 50  $\mu$ l PCR reaction mixture (200 $\mu$ M dNTPs final concentration) containing 1.2U of Taq polymerase  
10 in the buffer supplied by the manufacturer (Perkin-Elmer Corporation), and 1  $\mu$ M of primers, using a program consisting of 30 cycles of 94°C./2', 68°C./2', and 72°C./3', with a pre- and post-incubation of 95°C./5' and 72°C./10', respectively. PCR primers for human Y4 were  
15 designed against the human Y4 sequence in the third intracellular loop and carboxy terminal regions: 5'-CGCGTGTTCACAAGGGCACCTA-3' and 5'-TGCCACTTAGCCTCAGGGACCC-3', respectively.

20 The PCR products were run on a 1.5% agarose gel and transferred to charged nylon membranes (Zetaprobe GT, BioRad), and analyzed as Southern blots. Hybridization probes corresponding to the receptor region flanked by PCR primers were prepared (5' -  
25 TCCGTATGTACTGTGGACAGGGGCAGATGCTCCGACTCCTCCAGG-3') and pre-screened for the absence of cross-reactivity with human Y1 and human Y2 receptor subtypes. Filters were hybridized with end-labeled [ $\gamma$ -<sup>32</sup>P]ATP internal probe to the PCR primers, washed under high stringency, and  
30 exposed to Kodak XAR film in the presence of an intensifying screen, as described above. Similar PCR and Southern blot analysis were conducted with primers and probe directed to the housekeeping gene, glyceraldehyde-3-phosphate dehydrogenase (Clontech, Palo  
35 Alto, CA), and demonstrated that equal amounts of cDNA from the different tissues were being assayed for NPY expression.

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Results

A human genomic placenta library was screened, under  
5 reduced stringency conditions, with oligonucleotide  
probes directed to the first, second, third, fifth, and  
seventh transmembrane regions of the rat Y1 neuropeptide  
receptor gene (Eva, C. et al., 1990; GenBank accession  
No. Z11504). Positively-hybridizing clones ( $\approx$ 100-150)  
10 were isolated, plaque-purified and characterized by  
Southern blot analysis and sequencing. One clone, hp25a,  
contained a 1.3 kb PstI fragment which hybridized with  
the rat Y1-derived oligonucleotide probes and was  
subsequently subcloned into a pUC vector. DNA sequence  
15 analysis indicated greatest homology to the rat and human  
Y1 receptor genes. This clone was a partial intronless  
gene fragment, encoding part of the third intracellular  
loop through the carboxyl terminus, including a  
termination codon.

20

In order to obtain a full-length clone, a 2.0 kb  
BamHI/EcoRI hybridizing fragment, containing the entire  
coding region, which was intronless, was subcloned into  
an expression vector and sequenced. The genomic full-  
25 length construct in the expression vector (called  
hp25a/EXJ) contains an open reading frame of 1127 bp,  
with 680 bp of the predicted 5' UT and 205 bp of  
predicted 3' UT sequence, and encodes a protein of 375 aa  
in length, with a relative molecular mass of  $\approx$ 41,000  
30 daltons. Hydropathy analysis of the protein is  
consistent with a putative topography of seven  
transmembrane domains, indicative of the G protein-  
coupled receptor family.

35 Initial sequence analysis revealed that clone hp25a/EXJ  
contained several conserved structural features/residues  
found among the members of the neuropeptide receptor

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family, including two glycines and asparagine in TM1 (positions 55, 58 and 59, respectively, in Fig. 2), an asparagine, leucine and aspartic acid in TM2 (positions 82, 83, and 87, respectively, in Fig. 2), a serine and leucine in TM3 (positions 128 and 132, respectively, in Fig. 2), a tryptophan and proline in TM4 (positions 164 and 173, respectively, in Fig. 2), a tyrosine and proline in TM5 (positions 223 and 226, respectively, in Fig. 2), a phenylalanine, tryptophan, and proline in TM6 (positions 275, 279, and 281, respectively, in Fig. 2), and a serine, threonine, asparagine, and proline in TM7 (positions 315, 316, 319, and 320, respectively, in Fig. 2). Other features of this human hp25a receptor gene are the presence of three potential sites for N-linked glycosylation in the amino terminus (asparagine residues 2, 19, and 29; Fig. 1) and the presence of several serines and threonines in the carboxyl terminus and intracellular loops, which may serve as sites for potential phosphorylation by protein kinases.

20

A comparison of nucleotide and peptide sequences of clone hp25a/EXJ with sequences contained in the Genbank/EMBL databases reveals that the clone is most related to the rat, mouse and human Y1 receptor genes and proteins (see Fig. 2). The hp25a clone exhibits 42% overall amino acid identity with the human NPY-1 receptor and 55% identity when comparing only the transmembrane domains between hp25a and Y1. The comparison of the individual amino acid residues in the TM domains between hp25a and Y1 reveal <30%, 57%, 57%, 57%, 52%, 63%, and 71% identity in the corresponding one through seven TM regions, respectively. The hp25a clone hybridized only with the TM7-specific probe from the original set of rat-derived TM probes originally used to screen the library which is consistent with the hp25a clone sharing the highest degree of amino acid identity with the TM7 domain of the rat Y1 receptor.



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A rat homolog of the human Y4 receptor, designated rs16b, was isolated from a rat spleen genomic library using probes derived from the transmembrane regions of the human Y4 receptor. The nucleotide sequence of rs16b is 80% identical in the coding region to the nucleotide sequence of the human Y4 receptor, and encodes a protein 375 amino acids in length (Figure 3). The rs16b clone exhibits 75% overall amino acid identity with the human Y4 amino acid sequence, and in the putative transmembrane domains (TMs), the protein predicted by rs16b exhibits 84% amino acid identity with the human Y4 receptor. This degree of primary amino acid sequence identity is lower than is typically seen for species homologues, and suggests that rat and human Y4 receptors may exhibit functional variations as well. The predicted intracellular loop between TMs V and VI is particularly divergent, showing only 56% amino acid identity between rat and human Y4; divergence in this region could potentially mediate differences in G-protein coupling between the rat and human receptors. The primary sequences of rat and human Y4 receptors also show differences in their patterns of sequence motifs for casein kinase II phosphorylation, N-myristoylation, and protein kinase C phosphorylation; these sites could potentially mediate differences in the function or regulation of the two receptors.

Monkey kidney cells transiently expressing the gene encoding the hp25a receptor were used for pharmacological evaluation. Membranes harvested from transiently transfected Cos-7 cells exhibited high affinity, saturable [<sup>125</sup>I]PYY binding. The time course of specific binding was measured in the presence of 0.06 nM <sup>125</sup>I-PYY (Fig. 5). The association curve was monophasic, with an observed association rate ( $K_{obs}$ ) of  $0.12 \pm 0.02 \text{ min}^{-1}$  and a  $t_{1/2}$  of 6 min; equilibrium binding was 95% complete

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within 26 min, and 100% complete within 50 min ( $n = 3$ ). For comparison, we also measured the time course of binding to human Y1 receptors transiently expressed in COS-7 cells. The association curve was monophasic, with  
5 a  $K_{obs}$  of  $0.06 \pm 0.02 \text{ min}^{-1}$  and a  $t_{1/2}$  of 12 min; equilibrium binding was 95% complete within 51 min and 100% complete within 90 min ( $n = 3$ ) (data not shown). The different patterns of radioligand association for hp25a and human Y1 receptors suggest novel mechanisms of receptor/ligand  
10 interaction.

Saturation binding data for  $^{125}\text{I}$ -PYY were fit to a one-site model with an apparent  $K_d$  of  $0.11 \pm 0.01 \text{ nM}$  and an apparent  $B_{max}$  of  $1.42 \pm 0.05 \text{ pmol/mg}$  membrane protein,  
15 corresponding to approximately  $1.4 \times 10^5$  receptors/cell ( $n = 4$ ; Fig. 6). Given that the transfection efficiency was 20-30% (data not shown), the receptor density on transfected cells was probably closer to  $7 \times 10^5/\text{cell}$ . Membranes from mock-transfected cells, when prepared and  
20 analyzed in the same way as those from hp25a-transfected cells, displayed no specific binding of  $^{125}\text{I}$ -PYY. We conclude that the  $^{125}\text{I}$ -PYY binding sites observed under the described conditions were derived from the hp25a construct.

25

The pharmacological profile of hp25a was defined by membrane binding assays. The receptor was probed for features of all well characterized pancreatic polypeptide family receptors including Y1, Y2, Y3, and PP. The rank  
30 order of affinity for several peptide analogs was derived from competitive displacement of  $^{125}\text{I}$ -PYY (Fig. 7 and Table 2). The hp25a receptor was compared with two model systems: 1) the cloned human Y1 receptor (Larhammar et al., 1992; Herzog et al., 1992) transiently expressed in  
35 COS-7 cells, and 2) the Y2-like receptor population expressed by human SK-N-BE(2) neuroblastoma cells (Wahlestedt et al., 1991; Dumont et al., 1992). No

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models for human Y3 and human PP receptors have been described.

PP bound to hp25a with extremely high affinity ( $K_i = 0.029$  nM) and dramatic selectivity: PP was > 6000-fold selective for hp25a over human Y1 receptors ( $K_i = 200$  nM) and SK-N-Be(2) receptors ( $K_i > 300$  nM). This profile suggests that hp25a could function selectively as a PP receptor *in vivo*. The data further indicated, however, that hp25a bound quite well to human NPY ( $K_i = 1.4$  nM) and even better to human PYY ( $K_i = 0.62$  nM). These  $K_i$  values, while lower than the  $K_i$  for PP, are comparable to the effective concentrations of NPY and PYY from numerous physiological and pharmacological studies (Dumont, 1992). In our investigation, SK-N-Be(2) receptors bound human NPY and human PYY in the same rank order as hp25a but with 5- to 10-fold higher affinity, whereas human Y1 receptors bound human NPY and human PYY in the opposite rank order with 5- to 30-fold higher affinity. Hydrolysis of the carboxy terminal amide to free carboxylic acid, as in human NPY free acid, was disruptive for binding to all receptors. A requirement for a carboxy terminal amide appears to be a common structural feature of all pancreatic polypeptide family peptide/receptor interactions.

Fuhlendorff and co-workers replaced Ile<sup>31</sup> and Gln<sup>34</sup> in NPY with the corresponding residues from PP to create [Leu<sup>31</sup>,Pro<sup>34</sup>]NPY, which is commonly used to distinguish Y1 from Y2 receptors (Fuhlendorff, 1990). Human [Leu<sup>31</sup>,Pro<sup>34</sup>]NPY displayed > 2300-fold selectivity for human Y1 receptors over SK-N-Be(2), but only 5-fold selectivity for human Y1 receptors over hp25a. Human [Leu<sup>31</sup>,Pro<sup>34</sup>]NPY was a better ligand for hp25a ( $K_i = 0.60$  nM) than was human NPY itself ( $K_i = 1.4$  nM). This is possibly a reflection of the way in which [Leu<sup>31</sup>,Pro<sup>34</sup>]NPY mimics PP at positions 31 and 34. In contrast, the

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[Leu<sup>31</sup>, Pro<sup>34</sup>]NPY analog was well tolerated by the human Y1 receptor ( $K_i = 0.13$  nM), but not preferred over the parent peptide ( $K_i = 0.049$  nM).

5 hp25a displayed an intermediate level of sensitivity to N-terminal deletions of NPY and PYY, less so than human Y1 receptors. Removing Tyr<sup>1</sup> from porcine NPY resulted in a 29-fold loss in affinity for human Y1 receptors when compared with the full length parent peptide. The same  
10 modification decreased affinity 4-fold for hp25a receptors and 3-fold for SK-N-Be(2) receptors. It is interesting in this regard that human PP contains Ala<sup>1</sup>; the Tyr<sup>1</sup> of NPY may not play much of a role in receptor recognition. Truncation to NPY<sub>13-36</sub> decreased affinity  
15 1000-fold for human Y1 receptors, 33-fold for hp25a, and 4-fold for SK-N-Be(2) receptors. Further truncation to porcine NPY<sub>22-36</sub> decreased affinity 3500-fold for human Y1 receptors, 120-fold for hp25a, and 11-fold for SK-N-Be(2) receptors. In this regard, the hp25a receptor  
20 shares features of both Y1- and Y2-like pharmacology, as would be expected if the N-terminal region of porcine NPY were only moderately involved in receptor recognition.

An important structural difference between human PP,  
25 human PYY and human NPY is that both human NPY and PYY contain Gln<sup>34</sup>, whereas human PP contains Pro<sup>34</sup>. When Gln<sup>34</sup> in NPY was replaced with Pro<sup>34</sup> (as in the analog [Leu<sup>31</sup>, Pro<sup>34</sup>]NPY), an increase in binding affinity for the human Y4 receptor was observed. A similar increase in  
30 binding affinity was detected when Gln<sup>34</sup> of PYY was replaced with Pro<sup>34</sup>, supporting the proposal that PP-like peptides are preferred by the Y4 receptor. Replacement of Pro<sup>34</sup> in human PP by Gln<sup>34</sup> (as in [Ile<sup>31</sup>, Gln<sup>34</sup>]PP) caused very little change in PP binding affinity,  
35 however, suggesting that in the case of PP there are significant contributions to binding affinity from other regions of the peptide structure.

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Applicants further extended the structure/activity data for human PP fragments (PP<sub>2-36</sub>, PP<sub>13-36</sub>, PP<sub>20-36</sub>, PP<sub>27-36</sub>, and PP<sub>31-36</sub>). PP binding was unaffected by N-terminal truncation to PP<sub>2-36</sub>, but further truncation to PP<sub>13-36</sub> and beyond was disruptive. The shortest PP fragment tested, PP<sub>31-36</sub>, bound selectively to the Y4 receptor with  $K_i = 350$  nM, and hydrolysis of the C-terminal amide was detrimental ( $K_i > 10,000$  nM for human PP<sub>31-36</sub> free acid), as reported earlier for NPY. We conclude that the binding of PP to the Y4 receptor resembles the binding of NPY to the Y1 receptor, in that 1) Pro<sup>34</sup> is well-tolerated and 2) both ends of the peptide are required for optimal binding activity. This is in contrast to the Y2 binding model, in which 1) Pro<sup>34</sup> is not well-tolerated and 2) the N-terminal region of NPY does not contribute significantly to binding affinity. Note also that the Y2-selective ligands human PYY<sub>3-36</sub> and C2-NPY display relatively low affinity for the human Y4 receptor.

Additionally, the binding of the tetrapeptide invertebrate neurotransmitter Phe-Met-Arg-Phe-Amide (FMRF-amide) was investigated. This peptide has been shown to mimic several functions of NPY including the stimulation of food intake in rats (Robert, 1988). FMRFamide bound selectively to the Y4 receptor with a  $K_i$  value of 4000 nM. A closely related derivative, Phe-Leu-Arg-Phe-amide (FLRFamide), displayed improved Y4 binding affinity ( $K_i = 750$  nM) while maintaining selectivity. We also investigated the binding of [D-Trp<sup>32</sup>]NPY. This peptide was reported to stimulate food intake when injected into rat hypothalamus, and also to attenuate NPY-induced feeding in the same paradigm (Balasubramaniam, 1994). [D-Trp<sup>32</sup>]NPY displayed relatively low binding affinity for the human Y4 receptor as well as for the human Y1 and Y2 receptor subtypes. Data for these and other new peptides not included in the original patent filing are listed in Table 3.

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Untransfected NIH-3T3 and LM(tk-) were pre-screened for specific  $^{125}\text{I}$ -PYY binding and found to be negative (data not shown). After co-transfection with the human Y4 cDNA and a G418-resistant gene and selection with G-418, 5 surviving colonies were screened for specific binding of  $^{125}\text{I}$ -PYY. Two positive clones were identified and isolated for further study (NIH-3T3 hY4 clone #5 and LM(tk-) hY4 clone #3). The binding of  $^{125}\text{I}$ -PYY to membranes from the NIH-3T3 stable clone was saturable over a radioligand 10 concentration range of 0.5 pM to 2.5 nM. Binding data were fit to a one-site binding model with an apparent  $K_d$  of 0.17 nM  $\pm$  0.005 and a receptor density of 350  $\pm$  80 fmol/mg membrane protein (mean  $\pm$  s.e.m.,  $n = 2$ ). The LM(tk-) clone displayed an estimated receptor density of 15 7 fmol/mg membrane protein during the primary selection screen and was not analyzed further in a saturation assay.

Activation of all Y-type receptors described thus far is 20 thought to involve coupling to pertussis toxin-sensitive G-proteins which are inhibitory for adenylate cyclase activity ( $G_i$  or  $G_o$ ) (Wahlestedt and Reis, 1993). Based on these prior observations, we investigated the ability of PP to inhibit forskolin-stimulated cAMP accumulation in 25 LM(tk-) cells stably expressing the human Y4 receptor. Incubation of intact cells with 10  $\mu\text{M}$  forskolin produced ~10-fold increase in cAMP accumulation over a 5 minute period, as determined by radioimmunoassay. Simultaneous incubation with human PP decreased the forskolin- 30 stimulated cAMP accumulation by 67% in stably transfected LM(tk-) cells (Fig. 8) but not in untransfected cells (data not shown). Applicants conclude that human Y4 receptor activation can result in decreased cAMP accumulation, very likely through inhibition of adenylate 35 cyclase activity.

Peptides selected for their ability to bind to the

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transiently expressed human Y4 receptor were investigated for their ability to activate the human Y4 in the cAMP assay (Table 4). Note that both human PP and human PP<sub>2-36</sub> bound the Y4 receptor with a K<sub>i</sub> value of 0.06 nM, and that each displayed comparable activity in the cAMP assay with closely matching EC<sub>50</sub> values of 0.09 nM and 0.08 nM, respectively. The truncated PP fragments PP<sub>27-36</sub> and PP<sub>31-36</sub> were relatively weak ligands in the binding assay and were also less than 50% as effective as the full length PP in reducing forskolin-stimulated cAMP, thereby acting as partial agonists. Similarly, both NPY and PYY (which deviate from PP primarily in the N-terminal regions) yielded EC<sub>50</sub> values > 10-fold larger than their K<sub>i</sub> values. Receptor activation (more so than binding) may therefore depend heavily upon N-terminal PP structure. The functional activity of the reported feeding behavior modulator [D-Trp<sup>32</sup>]NPY was also investigated. Consistent with this peptide's low binding affinity for the human Y4 receptor, no functional activity of the peptide was detected at concentrations up to 0.3 uM (see Table 4), or when tested at 0.3 uM for antagonism of the PP functional response (data not shown).

The intracellular free calcium concentration was markedly increased in LM(tk-) cells stably transfected with the human Y4 receptor after application of 100 nM human PP ( $\Delta$  [Ca<sup>2+</sup>]<sub>i</sub> = 325 nM; Fig. 9). The response to 100 nM NPY was relatively small ( $\Delta$  [Ca<sup>2+</sup>]<sub>i</sub> = 68 nM). Untransfected LM(tk-) cells were unresponsive to either peptide (data not shown). When human PP was further analyzed in a concentration/response curve, the maximum  $\Delta$  [Ca<sup>2+</sup>]<sub>i</sub> measured was 334 nM and the EC<sub>50</sub> was 35 nM (Fig. 9, Inset). This greater activity of PP over NPY is consistent with the pharmacological profiles derived from both binding and cAMP assays described above. The calcium mobilization assay thereby provides a second pathway through which Y4 receptor activation can be

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measured.

Y4 mRNA was detected by PCR techniques in a broad range of human tissues. Relatively intense hybridization signals were detected in total brain, coronary artery, and ileum, suggesting a potential role for Y4 receptors in CNS function, cardiovascular regulation, and gastrointestinal physiology (Table 5).

- 10 The cDNA corresponding to the rat Y4 homolog was transiently expressed in COS-7 cells for membrane binding studies. The binding of  $^{125}\text{I}$ -PYY to the rat Y4 receptor was saturable over a radioligand concentration of 0.5 pM to 2.5 nM. Binding data were fit to a one-site model with an apparent  $K_d$  of 0.15 nM  $\pm$  0.005 and a receptor density of 275  $\pm$  3 fmol/mg membrane protein (mean  $\pm$  s.e.m., n = 2). As determined by using peptide analogs within the pancreatic polypeptide family, the rat Y4 pharmacological profile bears a resemblance to the human Y4 receptor; there are several interesting exceptions, however, including frog PP, salmon PP, human PP<sub>31-36</sub>, and avian PP, each of which discriminated ~10-fold between the rat and human receptor subtypes (Table 6). The differences may reflect the fact that PP is not well conserved among species relative to NPY and PYY; hence the species homologs of PP are likely to exhibit more variability in ligand binding.

- In summary, both the human Y4 receptor and the rat Y4 receptor displayed features unique among the neuropeptide receptors, exhibiting a profile which is divergent from their closest relatives, Y1 or Y2, in that each binds optimally to PP rather than to NPY or PYY (see Tables 1, 2 and 6). Unlike the Y1 and Y2 receptor models, the Y4 receptor appears to be a reasonable target for all three peptide ligands.



TABLE I

Pharmacologically defined receptors for NPY and related pancreatic polypeptides.

Rank orders of affinity are based on published reports of binding and functional data (Wahlestedt et al., 1991; Schwartz et al., 1990; Wahlestedt et al., 1993; Dumont et al., 1992). Missing peptides in the series reflect a lack of published information.

Receptor	Affinity (-pK <sub>i</sub> or -pEC <sub>50</sub> )						
	11 to 10	10 to 9	9 to 8	8 to 7	7 to 6	< 6	
Y1	NPY PYY [Leu <sup>31</sup> , Pro <sup>34</sup> ]N PY		NPY <sub>2-36</sub>	NPY <sub>13-36</sub>	PP		
Y2		PYY NPY NPY <sub>2-36</sub>	NPY <sub>13-36</sub>			[Leu <sup>31</sup> , Pro <sup>34</sup> ]N PY PP	
Y3		NPY	[Pro <sup>34</sup> ]NPY	NPY <sub>13-36</sub> PP		PYY	
PP	PP		[Leu <sup>31</sup> , Pro <sup>34</sup> ]N PY			NPY	

TABLE 2

Pharmacological profile of the hp25a receptor.

Binding data reflect competitive displacement of  $^{125}\text{I}$ -PYY from membranes of COS-7 cells transiently expressing hp25a receptors. Peptides were tested at concentrations ranging from 0.001 nM to 100 nM.  $\text{IC}_{50}$  values corresponding to 50% displacement were determined by nonlinear regression analysis and converted to  $K_i$  values according to the equation,  $K_i = \text{IC}_{50}/(1 + [\text{L}]/K_d)$ , where  $[\text{L}]$  is the  $^{125}\text{I}$ -PYY concentration and  $K_d$  is the equilibrium dissociation constant of  $^{125}\text{I}$ -PYY. The data shown are representative of at least two independent experiments.

Competitor	Human Y1, $K_i$ (nM)	hp25a, $K_i$ (nM)	SK-N-Be (2), $K_i$ (nM)
human PP	$200 \pm 68$	$0.029 \pm 0.006$	$> 300$
human [Leu <sup>31,Pro34</sup> ]NPY	$0.13 \pm 0.02$	$0.60 \pm 0.09$	$> 300$
human PPY	$0.085 \pm 0.021$	$0.62 \pm 0.15$	$0.11 \pm 0.02$
porcine NPY	$0.049 \pm 0.001$	$1.2 \pm 0.2$	$0.28 \pm 0.04$
human NPY	$0.049 \pm 0.009$	$1.4 \pm 0.1$	$0.13 \pm 0.02$
porcine NPY <sub>2-36</sub>	$1.4 \pm 0.2$	$4.4 \pm 1.3$	$0.41 \pm 0.09$
porcine NPY <sub>13-36</sub>	$51 \pm 16$	$39 \pm 5$	$1.8 \pm 0.4$
porcine PYY <sub>13-36</sub>	$32 \pm 7$	$47 \pm 6$	$0.86 \pm 0.14$
porcine NPY <sub>16-36</sub>	$45 \pm 4$	$54 \pm 2$	$5.0 \pm 0.5$
porcine NPY <sub>18-36</sub>	$28 \pm 5$	$63 \pm 7$	$2.1 \pm 0.5$
human NPY free acid	$> 300$	$79 \pm 17$	$280 \pm 120$
porcine NPY <sub>20-36</sub>	$62 \pm 6$	$100 \pm 20$	$3.1 \pm 0.6$
porcine NPY <sub>22-36</sub>	$170 \pm 30$	$140 \pm 63$	$3.2 \pm 0.6$
porcine NPY <sub>26-36</sub>	$> 300$	$> 300$	$70 \pm 7$

Table 3: human Y4 receptor vs. Y-type receptors cloned from human.

Binding data reflect competitive displacement of  $^{125}\text{I}$ -PYY from membranes of COS-7 cells transiently expressing human Y1, human Y2, and human Y4 receptors.  $\text{IC}_{50}$  values corresponding to 50% displacement were determined by nonlinear regression analysis and converted to  $K_i$  values according to the equation Chang-Prusoff equation,  $K_i = \text{IC}_{50}/(1 + [\text{L}]/K_d)$ , where  $[\text{L}]$  is the  $^{125}\text{I}$ -PYY concentration and  $K_d$  is the equilibrium dissociation constant of  $^{125}\text{I}$ -PYY. Any peptide not included in the original patent filing is referred to as a "new peptide".

Table 3

Peptide	Y1	Y2	Y4	Comments
PP, human	77	> 1000	0.06	
PP <sub>2-36</sub> , human	> 40	> 100	0.06	new peptide
PP <sub>13-36</sub> , human	> 100	> 100	39	new peptide
PP <sub>20-36</sub> , human	> 100	> 100	> 100	new peptide
PP <sub>27-36</sub> , human	> 100	> 100	> 88	new peptide
PP <sub>31-36</sub> , human	> 10000	> 10000	350	new peptide
PP <sub>31-36</sub> free acid, human	> 10000	> 10000	> 10000	new peptide
Phe-Met-Arg-Phe-Amide	12000	75000	4000	
Phe-Leu-Arg-Phe-Amide	15000	> 10000 0	750	new peptide
[Ile <sup>31</sup> , Gln <sup>34</sup> ]PP, human	> 86	20	0.09	new peptide
PP, bovine	240	> 820	0.05	new peptide
PP, rat	460	> 1000	0.18	new peptide

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Table 3 continued

Peptide	Y1	Y2	Y4	Comments
PP, salmon	0.20	0.17	3.2	new peptide
PP, avian	400	> 1000	7.0	new peptide
PP, frog	98	> 1000	61	new peptide
PYY, human	0.19	0.36	0.87	
PYY, porcine	0.14	0.35	1.3	new peptide
PYY <sub>3-36</sub> , human	45	0.70	14	new peptide
PYY <sub>13-36</sub> , porcine	33	1.5	46	
[Pro <sup>34</sup> ] PYY, human	0.14	> 310	0.12	new peptide
Peptide	Y1	Y2	Y4	Notes
NPY, human	0.08	0.74	2.2	
NPY, porcine	0.07	0.81	1.1	
Melanostatin (frog NPY)	0.07	0.87	1.2	new peptide
NPY <sub>2-36</sub> , human	3.6	2.0	16	new peptide
NPY <sub>2-36</sub> , porcine	2.4	1.2	5.6	
NPY <sub>13-36</sub> , porcine	70	2.5	38	

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Table 3 Continued

Peptide	Y1	Y2	Y4	Comments
NPY <sub>16-36</sub> , porcine	41	3.6	54	
NPY <sub>18-36</sub> , porcine	70	4.2	> 290	
NPY <sub>20-36</sub> , porcine	63	3.6	120	
NPY <sub>22-36</sub> , porcine	> 1000	18	> 990	
NPY <sub>26-36</sub> , porcine	> 1000	380	304	
[Leu <sup>31</sup> , Pro <sup>34</sup> ]NPY, human	0.15	> 120	1.1	
[Leu <sup>31</sup> , Pro <sup>34</sup> ]NPY, porcine	0.15	> 540	1.5	new peptide
O-Me-Tyr <sup>21</sup> - NPY, human	0.12	1.55	6.1	new peptide
NPY free acid, human	490	> 1000	> 1000	
NPY <sub>1-24</sub> amide, human	> 1000	> 1000	> 1000	new peptide
C2-NPY, porcine	73	3.5	120	new peptide
[D-Trp <sup>32</sup> ]NPY, human	> 1000	> 1000	> 1000	new peptide

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TABLE 4: Functional activation of the human Y4 receptor and inhibition of cAMP accumulation.

$K_i$  values were derived from binding assays as described in Table 3. Peptides were evaluated for binding affinity and then analyzed for functional activity. Functional data were derived from radioimmunoassay of cAMP accumulation in stably transfected LM(tk-) cells stimulated with 10  $\mu$ M forskolin. The maximum inhibition of cAMP accumulation relative to that produced by human PP ( $E_{max}$ ) and the concentration producing a half-maximal effect ( $EC_{50}$ ) were determined by nonlinear regression.

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Table 4

Peptide	Binding	Function	
	K <sub>i</sub> (nM)	EC <sub>50</sub> (nM)	E <sub>max</sub>
PP, human	0.06	0.09	100 %
PP <sub>2-36</sub> , human	0.06	0.08	101%
PP <sub>13-36</sub> , human	39	580	96%
PP <sub>27-36</sub> , human	> 88	3500	50 %
PP <sub>31-36</sub> , human	> 10000	89000	47 %
[Ile <sup>31</sup> ,Gln <sup>34</sup> ]PP , human	0.09	0.27	101%
salmon PP	3.2	110	96%
PYY, human	0.87	47	118%
[Pro <sup>34</sup> ]PYY, human	0.12	1.1	106%
NPY, human	2.2	20	98%
NPY, porcine	1.1	68	105%
NPY <sub>18-36</sub>	> 290	Not detected	
[Leu <sup>31</sup> ,Pro <sup>34</sup> ]N PY, human	1.1	35	105%
[Leu <sup>31</sup> ,Pro <sup>34</sup> ]N PY,porcine	1.5	26	111%



Table 4 continued

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Peptide	Binding	Function	
[D-Trp <sup>32</sup> ]NPY, human	> 1000	Not detected	.

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TABLE 5: Macrolocalization of Y4 receptor mRNA in human tissues by PCR.

Localization data reflect PCR-based amplification of human Y4 cDNA derived from mRNA extracts of human tissues. Southern blots of the PCR products were prepared and hybridized with <sup>32</sup>P-labeled oligonucleotide probes selective for Y-type receptor subtypes. The labeled products were recorded on X-ray film and the relative signal density was determined by visual inspection. In this rating scheme, + = faint signal, + + = moderate signal, + + + = intense signal.

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Table 5

Human tissues	human Y4 PCR product
total brain	+++
frontal brain	+
ventricle (heart)	+ +
atrium (heart)	+
thoracic aorta	+ +
coronary artery	+ + +
nasal mucosa	+
mesentery	+ +
stomach	+ +
ileum	+ + +
pancreas	not determined
liver	( - )
kidney	not determined
bladder	+
penis	+
testes	+
uterus (endometrium)	+ +
uterus (myometrium)	+

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TABLE 6: Pharmacological binding profile of the rat Y4 receptor vs. the human Y4 receptor.

Binding data reflect competitive displacement of  $^{125}\text{I}$ -PYY from membranes of COS-7 cells transiently expressing rat Y4 and human Y4 receptors.  $\text{IC}_{50}$  values corresponding to 50% displacement were determined by nonlinear regression analysis and converted to  $K_i$  values according to the equation Chang-Prusoff equation,  $K_i = \text{IC}_{50}/(1 + [\text{L}]/K_d)$ , where  $[\text{L}]$  is the  $^{125}\text{I}$ -PYY concentration and  $K_d$  is the equilibrium dissociation constant of  $^{125}\text{I}$ -PYY.

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Table 6

Peptide	Rat Y4	Human Y4
PP, human	0.12	0.06
PP, rat	0.20	0.18
PP, bovine	0.15	0.05
PP, frog	0.19	62
PP, salmon	0.36	3.2
PP <sub>31-36</sub> , human	20	350
PP, avian	> 82	7
PP <sub>31-36</sub> free acid, human	> 100	> 10000
PYY, porcine	0.58	1.3
NPY, human	1.7	2.2
NPY, porcine	1.8	1.1
NPY <sub>2-36</sub> , human	5	16
NPY <sub>13-36</sub> , porcine	135	38
[Leu <sup>31</sup> ,Pro <sup>34</sup> ]NPY, human	0.59	1.2
NPY free acid, human	> 1000	> 1000
C2-NPY, porcine	22	120
[D-Trp32]NPY, human	> 1000	> 1000

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Discussion

Applicants have cloned DNA representing a novel human neuropeptide Y/peptide YY/pancreatic polypeptide receptor (Y4) from human genomic DNA. Of all known G protein-coupled receptor sequences (EMBL/Genbank Data Base), the greatest homology was displayed between hp25a and the Y1 receptor genes (mouse--Eva et al., 1992; rat--Eva et al., 1990; and human--Larhammar et al., 1992). Comparison of the human hp25a deduced amino acid sequence with known G protein-coupled receptor sequences indicates the greatest concentration of identical amino acids to be in the transmembrane domains. In these TM regions, the percentage of identity for hp25a clone is 55% compared to human Y1, and less than 35% with other members of the peptide subfamily and other G protein-coupled receptor subfamilies. The alignment of this human hp25a sequence, relative to other G protein-coupled receptors or other members of the neuropeptide receptor subfamily, specifically human Y1, indicates a unique sequence, proving hp25a is a newly characterized receptor. The homology of hp25a to Y1 indicates that it is related to the NPY/PYY/PP family of receptors.

While the hp25a human receptor sequence exhibits higher overall and transmembrane identity to the rs16b rat Y4 receptor sequence than to other Y-type receptors such as the human Y1 receptor, the divergence between the rat Y4 and human Y4 sequences may contribute to the pharmacological differences between the two receptors. The isolation of the rat homologue of the Y4 receptor provides the means to compare the pharmacological properties of the rat and human Y4 receptors (see below) in relation to their observed differences in primary structures. These data will be critical to the design and testing of human therapeutic agents acting

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at these sites.

The unique pharmacological profile of the hp25a human Y4 receptor suggests that this receptor can serve as a novel target for the development of subtype selective ligands. The competitive displacement studies indicate that human PP is the preferred ligand for hp25a. The receptor also binds with high affinity to human NPY and human PYY, which share  $\geq 47\%$  amino acid identity with human PP. Affinity is enhanced by modifying NPY to closely resemble PP, as in [Leu<sup>31</sup>,Pro<sup>34</sup>]NPY. Decreased affinity for C-terminal fragments of NPY suggest that both N- and C-terminal regions of NPY contribute to hp25a receptor recognition. hp25a was less sensitive to N-terminal deletion of NPY than was the human Y1 receptor. One may speculate that both Y1 and hp25a share a common mechanism of peptide interaction which has been optimized for either NPY or PP, respectively.

The pharmacological data do not support classification of hp25a as a Y1 receptor, in which case it would display > 4000-fold selectivity for binding to human NPY over human PP (Table 2). Neither do the data support classification as a Y2 receptor, in which case it would tolerate N-terminal deletion of NPY but not exchange of Gln<sup>34</sup> for Pro<sup>34</sup> (Table 2). Finally, the data fails to support the classification of hp25a as a Y3 receptor, since it would be expected to display greater affinity for NPY than for PP or PYY (Wahlestedt et al., 1991). Therefore, applicants are designating the hp25a receptor as a Y4 receptor.

The additional data included here reflect an increased understanding of receptor ligand/interactions. Our further characterization of Y4 receptor pharmacology has indicated, for example, that the binding affinity

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for either human NPY ( $K_i = 2.2$  nM) or human PYY ( $K_i = 0.87$  nM) can be enhanced by conversion to human [Leu, Pro<sup>34</sup>]NPY ( $K_i = 1.1$  nM) or human [Pro<sup>34</sup>]PYY ( $K_i = 0.14$  nM). This information supports the importance of

5 Pro<sup>34</sup>- in the peptide pharmacophore and could potentially be incorporated into the design of metabolically stable nonpeptide ligands with Y<sub>4</sub>-selectivity. Additionally, the data prompt a re-evaluation of literature reports in which [Pro<sup>34</sup>]PYY is

10 described as a Y<sub>1</sub>-selective ligand. Our results indicate that [Pro<sup>34</sup>]PYY does not discriminate between the cloned human Y<sub>1</sub> and cloned human Y<sub>4</sub> receptor ( $K_i = 0.12$  and  $0.14$  nM, respectively) such that [Pro<sup>34</sup>]PYY cannot be used in isolation to define receptor

15 subtypes.

Other particularly interesting peptides include FMRF-amide, FLRF-amide, and [D-Trp<sup>32</sup>]NPY. FMRF-amide and [D-Trp<sup>32</sup>]NPY have both been shown to modulate food intake

20 in rats (get ref from George M ). While FMRF-amide and its derivative displayed some degree of Y<sub>4</sub>-selectivity (albeit relatively low affinity compared to human PP), [D-Trp<sup>32</sup>]NPY was essentially inactive at all Y-receptor subtypes studied. These profiles must be

25 considered as efforts are undertaken to validate the receptor mechanism of NPY-induced food intake. The tetrapeptide FLRF-amide has additional value as a starting point for the design of small nonpeptide compounds with Y<sub>4</sub> selectivity.

30

Applicants now have several Y<sub>4</sub> receptor expression systems from which to choose, each uniquely suited to different research questions. The transient expression system in COS-7, for example, allows one to generate

35 sufficient quantities of membranes for routine structure/activity relationship questions. Applicants can also produce mutant receptors by site-directed



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mutagenesis or other mutagenesis techniques and express them transiently in COS-7 for a comparison of pharmacological properties with those of the wild-type receptor. In this way, one can gain insight into  
5 receptor binding pockets, ligand binding domains, and mechanisms of activation. Whereas the transient expression system requires a new transfection for every cell or membrane harvest, the stable expression system offers the convenience of a single transfection step  
10 followed by routine passaging techniques. The stable system also offers the opportunity to select receptor density, which could be an important factor in evaluating the intrinsic activity of Y4 receptor ligands.

15 Applicants' characterization of the stably expressed Y4 receptor now shows definitively that the Y4 receptor can couple simultaneously to both cAMP regulation and calcium mobilization in a single cell type. The  $EC_{50}$   
20 for the calcium response is significantly higher than the  $EC_{50}$  for the cAMP response, suggesting that calcium mobilization may reflect promiscuous coupling of the receptor to G-protein other than that required for cyclase regulation. The functional assays allow one to  
25 assign agonist and antagonist activities to receptor selective compounds and thereby provide one with critical tools for drug design.

The question logically arises as to whether hp25a  
30 should be classified as a PP receptor. To applicants' knowledge, no human PP receptor has been described. One must therefore look to the rat PP receptor for comparison. The rat PP receptor bound PP and analogs in the same rank order as hp25a (PP > [Leu<sup>31</sup>,Pro<sup>34</sup>]NPY >  
35 NPY) (Schwartz et al., 1990). The rat PP receptor also appeared to bind both N- and C-terminal regions of the peptide ligand (Schwartz et al., 1987). A glaring

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discrepancy between hp25a and the rat PP receptor is that the latter displayed > 10,000-fold selectivity for PP over NPY (Schwartz et al., 1990).

In applicants' localization experiments Y4 mRNA was  
5 detected by PCR techniques in a broad range of human tissues. Relatively intense hybridization signals were detected in total brain, coronary artery, and ileum, suggesting a potential role for Y4 receptors in CNS function, cardiovascular regulation, and  
10 gastrointestinal physiology. This localization pattern is consistent with previously reported studies of PP-mediated effects at 1) brainstem sites (McTigue et al., 1993; Whitcomb et al., 1990), 2) on arterial blood pressure (Wager-Page et al., 1993a) and 3) on gastric  
15 acid secretion and gastrointestinal motility (McTigue et al., 1993; Wager-Page et al., 1993b). A more definitive localization of the Y4 receptor mRNA and receptor expression (i.e., whether on enterocytes, vascular smooth muscle cells, neurons, etc.) is  
20 attainable through *in situ* hybridization and receptor autoradiography techniques. There are to applicants' knowledge no published reports of PP receptor localization in human tissue as obtained through binding or functional studies. It may be informative,  
25 however, to compare the human Y4 macrolocalization data presented here with PP receptor characterization in the rat. PP receptors have been described, for example, in brainstem nuclei such as the area postrema, interpeduncular nucleus, dorsomedial nucleus, and the  
30 nucleus tractus solitarius (Whitcomb et al., 1990), consistent with the identification of Y4 mRNA in human brain. The PP receptors in rat brain stem are accessible to circulating PP, which is released upon vagal stimulation of the pancreas during feeding  
35 (Whitcomb et al., 1990). Activation of brainstem PP receptors inhibits further pancreatic secretion, increases gastric acid secretion, enhances gastric

-81-

motility, and increases gastric emptying time (Louie et al., 1985; McTigue and Rogers, 1993). A Y4 receptor antagonist then, would be expected to slow down gastric emptying time and potentially reduce meal size.

5

Given the similarities in pharmacologic profiles between the published PP receptor and the hp25a human Y4 receptor, it would be tempting to call hp25a the human PP receptor. Applicants believe that calling  
10 hp25a the human PP receptor, however, would be misleading. This is because the relatively compressed window of affinity for PP, PYY, and NPY ( $0.02 \text{ nM} \leq K_i \leq 1.5 \text{ nM}$ ) makes hp25a a potential target for all three peptide ligands. Future localization experiments may  
15 help resolve the relationship between hp25a and the PP receptor.

Applicants propose that hp25a be known as the Y4 receptor. The name is not biased toward any one member  
20 of the pancreatic polypeptide family. The "Y" has its roots in the original classification of Y1 and Y2 receptor subtypes (Wahlestedt et al., 1987). The letter reflects the conservation in pancreatic polypeptide family members of the C-terminal tyrosine,  
25 described as "Y" in the single letter amino acid code. Applicants note that the cloned human Y1 receptor was introduced by Larhammar and co-workers as a "human neuro peptide Y/peptide YY receptor of the Y1 type", with peptide ligands listed in rank order of affinity  
30 (Larhammar et al., 1992). Similarly, hp25a could be described as a human pancreatic polypeptide/peptide YY/neuro peptide Y receptor of the Y4 type.

hp25a is to applicants' knowledge the first "Y type"  
35 receptor to be cloned from a subtype family other than Y1. The reported Y3 receptor cloned from bovine brain (Rimland et al., 1991) was later described as having

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been misidentified (Jazin et al., 1993; Herzog et al., 1993). A Y2-like receptor (PR4) was cloned from drosophila and characterized using mammalian analogs of NPY (Li et al., 1992); however, the classification of  
5 this receptor is controversial. The receptor was relatively insensitive to NPY; concentrations ranging from 0.3 to 10  $\mu$ M were required to elicit calcium mobilization in oocytes injected with PR4 mRNA (Li et al., 1992). The receptor also displayed a rank order  
10 of potency for NPY analogs distinct from that observed in mammalian systems (Wahlestedt et al., 1993; Li et al., 1992). Furthermore, an NPY analog has not been isolated from drosophila (Wahlestedt et al., 1993). It is possible that an unidentified ligand in drosophila  
15 can activate PR4 more readily than NPY, and as such, the receptor may eventually be reclassified.

The cloning and expression of a Y4 (hp25a) receptor represents a major advance in the ability to analyze  
20 numerous physiological processes mediated by the pancreatic polypeptide family. Binding sites for PP, PYY, or NPY have a widespread anatomical distribution in peripheral targets such as neuromuscular junction, smooth muscle, stomach chief cells, intestinal  
25 enterocytes, kidney proximal tubule, and fat cells (Dumont et al., 1992; Castan et al., 1992). These receptors are therefore in a position to potentially regulate a variety of physiological functions including cognition, circadian rhythm, EEG synchronization, body  
30 temperature, blood pressure, locomotor activity, neuroendocrine release, sexual/reproductive behavior, feeding, sympathetic activation, sensory transmission, gastrointestinal function, intestinal secretion, renal absorption, and cardiovascular function (Wahlestedt et  
35 al., 1993).

Y4 receptors are an invaluable resource for drug

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design.. The pancreatic polypeptide family is potentially involved in several pathophysiological conditions including memory loss, depression, anxiety, epileptic seizure, pain, hypertension, locomotor  
5 problems, circadian rhythm disorders, eating/body weight disorders, sexual/reproductive disorders, nasal congestion, and diarrhea (Wahlestedt et al., 1993; Dumont et al., 1992). The available data implicate this receptor in the control of obesity and other  
10 disorders of feeding including bulimia and anorexia. The chemical synthesis of selective drugs not only for Y4 but for all "Y type" receptors will be greatly accelerated by preliminary screening against a homogeneous population of cloned human Y4 receptors.  
15 As more specific pharmacological tools become available for probing receptor function, additional therapeutic indications are likely to be discovered.

Applicants do not know whether hp25a represents the  
20 single Y4 receptor expressed in the human genome, or whether there exists a group of structurally related Y4 receptor subtypes. This is an issue which can be resolved using nucleotide sequences from Y4 receptor as the basis for *in situ* localization, antisense or  
25 "knockout" strategies, homology cloning, and related techniques. Such approaches will enable one to investigate the existence of potentially novel receptor subtypes with pharmacologic and therapeutic significance.

30

In conclusion, the primary structure of the proteins encoded by hp25a (Y4) gene and its homolog in the rat, as well as its unique pharmacological profile obtained for the Y4 receptor subtype, indicate that these genes  
35 represent a new pancreatic polypeptide receptor subfamily. Additional cloning efforts will be required to isolate additional members of this newly recognized

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neuropeptide receptor family.

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## SEQUENCE LISTING

## (1) GENERAL INFORMATION:

(i) APPLICANT: Bard, Jonathan A.  
Walker, Mary  
Branchek, Theresa  
Weinshank, Richard L.

(ii) TITLE OF INVENTION: DNA ENCODING A HUMAN NEUROPEPTIDE Y/PEPTIDE  
YY/PANCREATIC POLYPEPTIDE RECEPTOR (Y4) AND USES THEREOF

(iii) NUMBER OF SEQUENCES: 28

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## (v) COMPUTER READABLE FORM:

(A) MEDIUM TYPE: Floppy disk  
(B) COMPUTER: IBM PC compatible  
(C) OPERATING SYSTEM: PC-DOS/MS-DOS  
(D) SOFTWARE: PatentIn Release #1.0, Version #1.25

## (vi) CURRENT APPLICATION DATA:

(A) APPLICATION NUMBER:  
(B) FILING DATE:  
(C) CLASSIFICATION:

## (viii) ATTORNEY/AGENT INFORMATION:

(A) NAME: White, John P.  
(B) REGISTRATION NUMBER: 28,678  
(C) REFERENCE/DOCKET NUMBER: 44743-A-PCT\JPW\MAT

## (ix) TELECOMMUNICATION INFORMATION:

(A) TELEPHONE: (212) 278-0400  
(B) TELEFAX: (212) 391-0525

## (2) INFORMATION FOR SEQ ID NO:1:

## (i) SEQUENCE CHARACTERISTICS:

(A) LENGTH: 1320 base pairs  
(B) TYPE: nucleic acid  
(C) STRANDEDNESS: single  
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: DNA (genomic)

(iii) HYPOTHETICAL: NO

(iv) ANTI-SENSE: NO

## (ix) FEATURE:

(A) NAME/KEY: CDS  
(B) LOCATION: 88..1212

## (xi) SEQUENCE DESCRIPTION: SEQ ID NO:1:

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Met Asn Thr Ser His Leu Leu Ala
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TTG	CTG	CTC	CCA	AAA	TCT	CCA	CAA	GGT	GAA	AAC	AGA	AGC	AAA	CCC	CTG	159														
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CTC	ATG	TGC	CTC	CTC	TGC	CAG	CCG	CTG	ACC	GCC	GTC	TAC	ACC	ATC	ATG	399														
Leu	Met	Cys	Leu	Leu	Cys	Gln	Pro	Leu	Thr	Ala	Val	Tyr	Thr	Ile	Met															
	90					95					100																			
GAC	TAC	TGG	ATC	TTT	GGA	GAG	ACC	CTC	TGC	AAG	ATG	TCG	GCC	TTC	ATC	447														
Asp	Tyr	Trp	Ile	Phe	Gly	Glu	Thr	Leu	Cys	Lys	Met	Ser	Ala	Phe	Ile															
105					110					115					120															
CAG	TGC	ATG	TCG	GTG	ACG	GTC	TCC	ATC	CTC	TCG	CTC	GTC	CTC	GTG	GCC	495														
Gln	Cys	Met	Ser	Val	Thr	Val	Ser	Ile	Leu	Ser	Leu	Val	Leu	Val	Ala															
				125					130					135																
CTG	GAG	AGG	CAT	CAG	CTC	ATC	ATC	AAC	CCA	ACA	GGC	TGG	AAG	CCC	AGC	543														
Leu	Glu	Arg	His	Gln	Leu	Ile	Ile	Asn	Pro	Thr	Gly	Trp	Lys	Pro	Ser															
			140					145					150																	
ATC	TCA	CAG	GCC	TAC	CTG	GGG	ATT	GTG	CTC	ATC	TGG	GTC	ATT	GCC	TGT	591														
Ile	Ser	Gln	Ala	Tyr	Leu	Gly	Ile	Val	Leu	Ile	Trp	Val	Ile	Ala	Cys															
		155					160					165																		
GTC	CTC	TCC	CTG	CCC	TTC	CTG	GCC	AAC	AGC	ATC	CTG	GAG	AAT	GTC	TTC	639														
Val	Leu	Ser	Leu	Pro	Phe	Leu	Ala	Asn	Ser	Ile	Leu	Glu	Asn	Val	Phe															
	170					175					180																			
CAC	AAG	AAC	CAC	TCC	AAG	GCT	CTG	GAG	TTC	CTG	GCA	GAT	AAG	GTG	GTC	687														
His	Lys	Asn	His	Ser	Lys	Ala	Leu	Glu	Phe	Leu	Ala	Asp	Lys	Val	Val															
					190					195				200																
TGT	ACC	GAG	TCC	TGG	CCA	CTG	GCT	CAC	CAC	CGC	ACC	ATC	TAC	ACC	ACC	735														
Cys	Thr	Glu	Ser	Trp	Pro	Leu	Ala	His	His	Arg	Thr	Ile	Tyr	Thr	Thr															
				205					210					215																
TTC	CTG	CTC	CTC	TTC	CAG	TAC	TGC	CTC	CCA	CTG	GGC	TTC	ATC	CTG	GTC	783														
Phe	Leu	Leu	Leu	Phe	Gln	Tyr	Cys	Leu	Pro	Leu	Gly	Phe	Ile	Leu	Val															
			220					225					230																	
TGT	TAT	GCA	CGC	ATC	TAC	CGG	CGC	CTG	CAG	AGG	CAG	GGG	CGC	GTG	TTT	831														
Cys	Tyr	Ala	Arg	Ile	Tyr	Arg	Arg	Leu	Gln	Arg	Gln	Gly	Arg	Val	Phe															
		235				240						245																		
CAC	AAG	GGC	ACC	TAC	AGC	TTG	CGA	GCT	GGG	CAC	ATG	AAG	CAG	GTC	AAT	879														
His	Lys	Gly	Thr	Tyr	Ser	Leu	Arg	Ala	Gly	His	Met	Lys	Gln	Val	Asn															
	250					255					260																			
GTG	GTG	CTG	GTG	GTG	ATG	GTG	GTG	GCC	TTT	GCC	GTG	CTC	TGG	CTG	CCT	927														
Val	Val	Leu	Val	Val	Met	Val	Val	Ala	Phe	Ala	Val	Leu	Trp	Leu	Pro															
	265				270					275					280															

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CTG CAT GTG TTC AAC AGC CTG GAA GAC TGG CAC CAT GAG GCC ATC CCC	975
Leu His Val Phe Asn Ser Leu Glu Asp Trp His His Glu Ala Ile Pro	
285 290 295	
ATC TGC CAC GGG AAC CTC ATC TTC TTA GTG TGC CAC TTG CTT GCC ATG	1023
Ile Cys His Gly Asn Leu Ile Phe Leu Val Cys His Leu Leu Ala Met	
300 305 310	
GCC TCC ACC TGC GTC AAC CCA TTC ATC TAT GGC TTT CTC AAC ACC AAC	1071
Ala Ser Thr Cys Val Asn Pro Phe Ile Tyr Gly Phe Leu Asn Thr Asn	
315 320 325	
TTC AAG AAG GAG ATC AAG GCC CTG GTG CTG ACT TGC CAG CAG AGC GCC	1119
Phe Lys Lys Glu Ile Lys Ala Leu Val Leu Thr Cys Gln Gln Ser Ala	
330 335 340	
CCC CTG GAG GAG TCG GAG CAT CTG CCC CTG TCC ACA GTA CAT ACG GAA	1167
Pro Leu Glu Glu Ser Glu His Leu Pro Leu Ser Thr Val His Thr Glu	
345 350 355 360	
GTC TCC AAA GGG TCC CTG AGG CTA AGT GGC AGG TCC AAT CCC ATT	1212
Val Ser Lys Gly Ser Leu Arg Leu Ser Gly Arg Ser Asn Pro Ile	
365 370 375	
TAACCAGGTC TAGGTCTTCT CCCTGCCATG TCCCTTGCCA GGCTCTTCCA CTTAGCTAAG	1272
TGGGCACACT GCAAGCTGGG GTGGCACCCC AGCATTCTG GCTTTCTG	1320

## (2) INFORMATION FOR SEQ ID NO:2:

## (i) SEQUENCE CHARACTERISTICS:

(A) LENGTH: 375 amino acids

(B) TYPE: amino acid

(D) TOPOLOGY: linear

## (ii) MOLECULE TYPE: protein

## (xi) SEQUENCE DESCRIPTION: SEQ ID NO:2:

Met Asn Thr Ser His Leu Leu Ala Leu Leu Leu Pro Lys Ser Pro Gln	
1 5 10 15	
Gly Glu Asn Arg Ser Lys Pro Leu Gly Thr Pro Tyr Asn Phe Ser Glu	
20 25 30	
His Cys Gln Asp Ser Val Asp Val Met Val Phe Ile Val Thr Ser Tyr	
35 40 45	
Ser Ile Glu Thr Val Val Gly Val Leu Gly Asn Leu Cys Leu Met Cys	
50 55 60	
Val Thr Val Arg Gln Lys Glu Lys Ala Asn Val Thr Asn Leu Leu Ile	
65 70 75 80	
Ala Asn Leu Ala Phe Ser Asp Phe Leu Met Cys Leu Leu Cys Gln Pro	
85 90 95	
Leu Thr Ala Val Tyr Thr Ile Met Asp Tyr Trp Ile Phe Gly Glu Thr	
100 105 110	
Leu Cys Lys Met Ser Ala Phe Ile Gln Cys Met Ser Val Thr Val Ser	
115 120 125	
Ile Leu Ser Leu Val Leu Val Ala Leu Glu Arg His Gln Leu Ile Ile	
130 135 140	
Asn Pro Thr Gly Trp Lys Pro Ser Ile Ser Gln Ala Tyr Leu Gly Ile	
145 150 155 160	

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Val Leu Ile Trp Val Ile Ala Cys Val Leu Ser Leu Pro Phe Leu Ala  
165 170 175

Asn Ser Ile Leu Glu Asn Val Phe His Lys Asn His Ser Lys Ala Leu  
180 185 190

Glu Phe Leu Ala Asp Lys Val Val Cys Thr Glu Ser Trp Pro Leu Ala  
195 200 205

His His Arg Thr Ile Tyr Thr Thr Phe Leu Leu Leu Phe Gln Tyr Cys  
210 215 220

Leu Pro Leu Gly Phe Ile Leu Val Cys Tyr Ala Arg Ile Tyr Arg Arg  
225 230 235 240

Leu Gln Arg Gln Gly Arg Val Phe His Lys Gly Thr Tyr Ser Leu Arg  
245 250 255

Ala Gly His Met Lys Gln Val Asn Val Val Leu Val Val Met Val Val  
260 265 270

Ala Phe Ala Val Leu Trp Leu Pro Leu His Val Phe Asn Ser Leu Glu  
275 280 285

Asp Trp His His Glu Ala Ile Pro Ile Cys His Gly Asn Leu Ile Phe  
290 295 300

Leu Val Cys His Leu Leu Ala Met Ala Ser Thr Cys Val Asn Pro Phe  
305 310 315 320

Ile Tyr Gly Phe Leu Asn Thr Asn Phe Lys Lys Glu Ile Lys Ala Leu  
325 330 335

Val Leu Thr Cys Gln Gln Ser Ala Pro Leu Glu Glu Ser Glu His Leu  
340 345 350

Pro Leu Ser Thr Val His Thr Glu Val Ser Lys Gly Ser Leu Arg Leu  
355 360 365

Ser Gly Arg Ser Asn Pro Ile  
370 375

## (2) INFORMATION FOR SEQ ID NO:3:

## (i) SEQUENCE CHARACTERISTICS:

- (A) LENGTH: 45 base pairs
- (B) TYPE: nucleic acid
- (C) STRANDEDNESS: single
- (D) TOPOLOGY: linear

(ii) MOLECULE TYPE: DNA (genomic)

(iii) HYPOTHETICAL: NO

(iv) ANTI-SENSE: NO

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:3:

TTGCTTATGG GGCTGTGATT ATTCTTGGGG TCTCTGGAAA CCTGG

45

## (2) INFORMATION FOR SEQ ID NO:4:

## (i) SEQUENCE CHARACTERISTICS:

- (A) LENGTH: 45 base pairs
- (B) TYPE: nucleic acid
- (C) STRANDEDNESS: single
- (D) TOPOLOGY: linear

(ii) MOLECULE TYPE: DNA (genomic)

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(iii) HYPOTHETICAL: NO

(iv) ANTI-SENSE: YES

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:4:

TAGGATGATT ATGATCAATG CCAGGTTTCC AGAGACCCCA AGAAT

45

(2) INFORMATION FOR SEQ ID NO:5:

(i) SEQUENCE CHARACTERISTICS:

(A) LENGTH: 45 base pairs

(B) TYPE: nucleic acid

(C) STRANDEDNESS: single

(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: DNA (genomic)

(iii) HYPOTHETICAL: NO

(iv) ANTI-SENSE: NO

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:5:

AAAGAGATGA GGAATGTCAC CAACATTCTG ATCGTGAACC TCTCC

45

(2) INFORMATION FOR SEQ ID NO:6:

(i) SEQUENCE CHARACTERISTICS:

(A) LENGTH: 45 base pairs

(B) TYPE: nucleic acid

(C) STRANDEDNESS: single

(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: DNA (genomic)

(iii) HYPOTHETICAL: NO

(iv) ANTI-SENSE: YES

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:6:

CAGCAAGTCT GAGAAGGAGA GGTTCACGAT CAGAATGTTG GTGAC

45

(2) INFORMATION FOR SEQ ID NO:7:

(i) SEQUENCE CHARACTERISTICS:

(A) LENGTH: 54 base pairs

(B) TYPE: nucleic acid

(C) STRANDEDNESS: single

(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: DNA (genomic)

(iii) HYPOTHETICAL: NO

(iv) ANTI-SENSE: NO

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:7:

TGCAAACTGA ATCCTTTTGT GCAATGCGTC TCCATTACAG TATCCATTTT CTCT

54

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## (2) INFORMATION FOR SEQ ID NO:8:

- (i) SEQUENCE CHARACTERISTICS:
  - (A) LENGTH: 54 base pairs
  - (B) TYPE: nucleic acid
  - (C) STRANDEDNESS: single
  - (D) TOPOLOGY: linear

(ii) MOLECULE TYPE: DNA (genomic)

(iii) HYPOTHETICAL: NO

(iv) ANTI-SENSE: YES

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:8:

ACGTTCCACA GCGATGAGAA CCAGAGAGAA AATGGATACT GTAATGGAGA CGCA

54

## (2) INFORMATION FOR SEQ ID NO:9:

- (i) SEQUENCE CHARACTERISTICS:
  - (A) LENGTH: 42 base pairs
  - (B) TYPE: nucleic acid
  - (C) STRANDEDNESS: single
  - (D) TOPOLOGY: linear

(ii) MOLECULE TYPE: DNA (genomic)

(iii) HYPOTHETICAL: NO

(iv) ANTI-SENSE: NO

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:9:

CTGCAGTATT TTGGCCCACT CTGTTTCATA TTCATATGCT AC

42

## (2) INFORMATION FOR SEQ ID NO:10:

- (i) SEQUENCE CHARACTERISTICS:
  - (A) LENGTH: 42 base pairs
  - (B) TYPE: nucleic acid
  - (C) STRANDEDNESS: single
  - (D) TOPOLOGY: linear

(ii) MOLECULE TYPE: DNA (genomic)

(iii) HYPOTHETICAL: NO

(iv) ANTI-SENSE: YES

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:10:

CAAGCGAATG TATATCTTGA AGTAGCATAT GAATATGAAA CA

42

## (2) INFORMATION FOR SEQ ID NO:11:

- (i) SEQUENCE CHARACTERISTICS:
  - (A) LENGTH: 48 base pairs
  - (B) TYPE: nucleic acid
  - (C) STRANDEDNESS: single
  - (D) TOPOLOGY: linear

(ii) MOLECULE TYPE: DNA (genomic)

(iii) HYPOTHETICAL: NO

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(iv) ANTI-SENSE: NO

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:11:

CTGCTCTGCC ACCTCACGGC CATGATCTCC ACCTGCGTCA ACCCCATC

48

(2) INFORMATION FOR SEQ ID NO:12:

(i) SEQUENCE CHARACTERISTICS:

(A) LENGTH: 48 base pairs

(B) TYPE: nucleic acid

(C) STRANDEDNESS: single

(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: DNA (genomic)

(iii) HYPOTHETICAL: NO

(iv) ANTI-SENSE: YES

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:12:

GAAATTTTTC TTCAGGAATC CATAAAAGAT GGGGTTGACG CAGGTGGA

48

(2) INFORMATION FOR SEQ ID NO:13:

(i) SEQUENCE CHARACTERISTICS:

(A) LENGTH: 47 base pairs

(B) TYPE: nucleic acid

(C) STRANDEDNESS: single

(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: DNA (genomic)

(iii) HYPOTHETICAL: NO

(iv) ANTI-SENSE: NO

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:13:

TCATCGTCAC TTCCTACAGC ATTGAGACTG TCGTGGGGGT CCTGGGT

47

(2) INFORMATION FOR SEQ ID NO:14:

(i) SEQUENCE CHARACTERISTICS:

(A) LENGTH: 46 base pairs

(B) TYPE: nucleic acid

(C) STRANDEDNESS: single

(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: DNA (genomic)

(iii) HYPOTHETICAL: NO

(iv) ANTI-SENSE: NO

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:14:

ACAGTCACAC ACATCAGGCA GAGGTTACCC AGGACCCCCA CGACAG

46

(2) INFORMATION FOR SEQ ID NO:15:

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- (i) SEQUENCE CHARACTERISTICS:
  - (A) LENGTH: 45 base pairs
  - (B) TYPE: nucleic acid
  - (C) STRANDEDNESS: single
  - (D) TOPOLOGY: linear

- (ii) MOLECULE TYPE: DNA (genomic)

- (iii) HYPOTHETICAL: NO

- (iv) ANTI-SENSE: NO

- (xi) SEQUENCE DESCRIPTION: SEQ ID NO:15:

TGCTTATCGC CAACCTGGCC TTCTCTGACT TCCTCATGTG CCTCC

45

- (2) INFORMATION FOR SEQ ID NO:16:

- (i) SEQUENCE CHARACTERISTICS:
  - (A) LENGTH: 45 base pairs
  - (B) TYPE: nucleic acid
  - (C) STRANDEDNESS: single
  - (D) TOPOLOGY: linear

- (ii) MOLECULE TYPE: DNA (genomic)

- (iii) HYPOTHETICAL: NO

- (iv) ANTI-SENSE: NO

- (xi) SEQUENCE DESCRIPTION: SEQ ID NO:16:

TAGACGGCGG TCAGCGGCTG GCAGAGGAGG CACATGAGGA AGTCA

45

- (2) INFORMATION FOR SEQ ID NO:17:

- (i) SEQUENCE CHARACTERISTICS:
  - (A) LENGTH: 45 base pairs
  - (B) TYPE: nucleic acid
  - (C) STRANDEDNESS: single
  - (D) TOPOLOGY: linear

- (ii) MOLECULE TYPE: DNA (genomic)

- (iii) HYPOTHETICAL: NO

- (iv) ANTI-SENSE: NO

- (xi) SEQUENCE DESCRIPTION: SEQ ID NO:17:

TGTCGGCCTT CATCCAGTGC ATGTCGGTGA CGGTCTCCAT CCTCT

45

- (2) INFORMATION FOR SEQ ID NO:18:

- (i) SEQUENCE CHARACTERISTICS:
  - (A) LENGTH: 45 base pairs
  - (B) TYPE: nucleic acid
  - (C) STRANDEDNESS: single
  - (D) TOPOLOGY: linear

- (ii) MOLECULE TYPE: DNA (genomic)

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(iii) HYPOTHETICAL: NO

(iv) ANTI-SENSE: NO

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:18:

CTCTCCAGGG CCACGAGGAC GAGCGAGAGG ATGGAGACCG TCACC

45

(2) INFORMATION FOR SEQ ID NO:19:

- (i) SEQUENCE CHARACTERISTICS:
  - (A) LENGTH: 45 base pairs
  - (B) TYPE: nucleic acid
  - (C) STRANDEDNESS: single
  - (D) TOPOLOGY: linear

(ii) MOLECULE TYPE: DNA (genomic)

(iii) HYPOTHETICAL: NO

(iv) ANTI-SENSE: NO

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:19:

GCCTACCTGG GGATTGTGCT CATCTGGGTC ATTGCCTGTG TCCTC

45

(2) INFORMATION FOR SEQ ID NO:20:

- (i) SEQUENCE CHARACTERISTICS:
  - (A) LENGTH: 45 base pairs
  - (B) TYPE: nucleic acid
  - (C) STRANDEDNESS: single
  - (D) TOPOLOGY: linear

(ii) MOLECULE TYPE: DNA (genomic)

(iii) HYPOTHETICAL: NO

(iv) ANTI-SENSE: NO

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:20:

TGCTGTTGGC CAGGAAGGGC AGGGAGAGGA CACAGGCAAT GACCC

45

(2) INFORMATION FOR SEQ ID NO:21:

- (i) SEQUENCE CHARACTERISTICS:
  - (A) LENGTH: 45 base pairs
  - (B) TYPE: nucleic acid
  - (C) STRANDEDNESS: single
  - (D) TOPOLOGY: linear

(ii) MOLECULE TYPE: DNA (genomic)

(iii) HYPOTHETICAL: NO

(iv) ANTI-SENSE: NO

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:21:



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CATCTACACC ACCTTCCTGC TCCTCTTCCA GTACTGCCTC CCACT

45

## (2) INFORMATION FOR SEQ ID NO:22:

- (i) SEQUENCE CHARACTERISTICS:
  - (A) LENGTH: 45 base pairs
  - (B) TYPE: nucleic acid
  - (C) STRANDEDNESS: single
  - (D) TOPOLOGY: linear

(ii) MOLECULE TYPE: DNA (genomic)

(iii) HYPOTHETICAL: NO

(iv) ANTI-SENSE: NO

## (xi) SEQUENCE DESCRIPTION: SEQ ID NO:22:

TGCATAACAG ACCAGGATGA AGCCCAGTGG GAGGCAGTAC TGGAA

45

## (2) INFORMATION FOR SEQ ID NO:23:

- (i) SEQUENCE CHARACTERISTICS:
  - (A) LENGTH: 46 base pairs
  - (B) TYPE: nucleic acid
  - (C) STRANDEDNESS: single
  - (D) TOPOLOGY: linear

(ii) MOLECULE TYPE: DNA (genomic)

(iii) HYPOTHETICAL: NO

(iv) ANTI-SENSE: NO

## (xi) SEQUENCE DESCRIPTION: SEQ ID NO:23:

CTGGTGGTGA TGGTGGTGGC CTTTGCCGTG CTCTGGCTGC CTCTGC

46

## (2) INFORMATION FOR SEQ ID NO:24:

- (i) SEQUENCE CHARACTERISTICS:
  - (A) LENGTH: 45 base pairs
  - (B) TYPE: nucleic acid
  - (C) STRANDEDNESS: single
  - (D) TOPOLOGY: linear

(ii) MOLECULE TYPE: DNA (genomic)

(iii) HYPOTHETICAL: NO

(iv) ANTI-SENSE: NO

## (xi) SEQUENCE DESCRIPTION: SEQ ID NO:24:

CAGTCTTCCA GGCTGTTGAA CACATGCAGA GGCAGCCAGA GCACG

45

## (2) INFORMATION FOR SEQ ID NO:25:

- (i) SEQUENCE CHARACTERISTICS:
  - (A) LENGTH: 45 base pairs
  - (B) TYPE: nucleic acid

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(C) STRANDEDNESS: single  
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: DNA (genomic)  
(iii) HYPOTHETICAL: NO  
(iv) ANTI-SENSE: NO

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:25:

ATCTTCTTAG TGTGCCACTT GCTTGCCATG GCCTCCACCT GCGTC

45

(2) INFORMATION FOR SEQ ID NO:26:

(i) SEQUENCE CHARACTERISTICS:  
(A) LENGTH: 45 base pairs  
(B) TYPE: nucleic acid  
(C) STRANDEDNESS: single  
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: DNA (genomic)  
(iii) HYPOTHETICAL: NO  
(iv) ANTI-SENSE: NO

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:26:

TGAGAAAGCC ATAGATGAAT GGGTTGACGC AGGTGGAGGC CATGG

45

(2) INFORMATION FOR SEQ ID NO:27:

(i) SEQUENCE CHARACTERISTICS:  
(A) LENGTH: 1439 base pairs  
(B) TYPE: nucleic acid  
(C) STRANDEDNESS: single  
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: DNA (genomic)  
(iii) HYPOTHETICAL: NO  
(iv) ANTI-SENSE: NO

(ix) FEATURE:  
(A) NAME/KEY: CDS  
(B) LOCATION: 178..1306

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:27:

ATAGCTCTCA AGCCATAAGA TATAAGTAGC TAAGAATTGT CTCCCTCTCC CTGTCCCTTG	60
TTCTTACCTG GTTCCATTTT ACATGCCTGG ACCTTTGAGT TCCATTGTGT TGTTTTGCAG	120
GCTACACTCA GAAGTGGGCC CTTTAGTCTT GAAGTTCCTG GTCTTCTCAC ACCCACC	177
ATG AAT ACC TCT CAT CTC ATG GCC TCC CTT TCT CCG GCA TTC CTA CAA	225
Met Asn Thr Ser His Leu Met Ala Ser Leu Ser Pro Ala Phe Leu Gln	
1 5 10 15	
GGT AAG AAT GGG ACC AAC CCA CTG GAT TCC CTC TAT AAT CTC TCT GAC	273

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Gly	Lys	Asn	Gly	Thr	Asn	Pro	Leu	Asp	Ser	Leu	Tyr	Asn	Leu	Ser	Asp	
			20					25					30			
GGC	TGC	CAG	GAT	TCG	GCA	GAT	CTG	TTG	GCC	TTC	ATC	ATC	ACC	ACC	TAC	321
Gly	Cys	Gln	Asp	Ser	Ala	Asp	Leu	Leu	Ala	Phe	Ile	Ile	Thr	Thr	Tyr	
		35					40				45					
AGC	GTT	GAG	ACC	GTC	TTG	GGG	GTC	CTA	GGA	AAC	CTC	TGC	TTG	ATA	TTT	369
Ser	Val	Glu	Thr	Val	Leu	Gly	Val	Leu	Gly	Asn	Leu	Cys	Leu	Ile	Phe	
	50					55					60					
GTG	ACC	ACA	AGG	CAA	AAG	GAA	AAG	TCC	AAT	GTG	ACC	AAC	CTA	CTC	ATT	417
Val	Thr	Thr	Arg	Gln	Lys	Glu	Lys	Ser	Asn	Val	Thr	Asn	Leu	Leu	Ile	
	65				70					75					80	
GCC	AAC	CTG	GCC	TTC	TCT	GAC	TTC	CTC	ATG	TGT	CTC	ATC	TGC	CAG	CCG	465
Ala	Asn	Leu	Ala	Phe	Ser	Asp	Phe	Leu	Met	Cys	Leu	Ile	Cys	Gln	Pro	
				85					90					95		
CTC	ACG	GTC	ACC	TAC	ACC	ATC	ATG	GAC	TAC	TGG	ATC	TTC	GGC	GAA	GTC	513
Leu	Thr	Val	Thr	Tyr	Thr	Ile	Met	Asp	Tyr	Trp	Ile	Phe	Gly	Glu	Val	
			100					105					110			
CTT	TGC	AAG	ATG	TTA	ACG	TTC	ATC	CAG	TGT	ATG	TCG	GTG	ACA	GTC	TCC	561
Leu	Cys	Lys	Met	Leu	Thr	Phe	Ile	Gln	Cys	Met	Ser	Val	Thr	Val	Ser	
		115					120					125				
ATC	CTC	TCA	CTG	GTC	CTT	GTG	GCC	CTG	GAG	AGG	CAC	CAG	CTC	ATT	ATC	609
Ile	Leu	Ser	Leu	Val	Leu	Val	Ala	Leu	Glu	Arg	His	Gln	Leu	Ile	Ile	
	130					135					140					
AAC	CCG	ACT	GGC	TGG	AAA	CCC	AGC	ATT	TCC	CAG	GCC	TAC	CTG	GGG	ATT	657
Asn	Pro	Thr	Gly	Trp	Lys	Pro	Ser	Ile	Ser	Gln	Ala	Tyr	Leu	Gly	Ile	
	145				150					155				160		
GTG	GTC	ATC	TGG	TTC	ATT	TCT	TGT	TTC	CTC	TCC	TTG	CCC	TTC	CTG	GCC	705
Val	Val	Ile	Trp	Phe	Ile	Ser	Cys	Phe	Leu	Ser	Leu	Pro	Phe	Leu	Ala	
			165					170						175		
AAT	AGC	ATC	CTG	AAC	GAC	CTC	TTC	CAC	TAC	AAC	CAC	TCT	AAG	GTT	GTG	753
Asn	Ser	Ile	Leu	Asn	Asp	Leu	Phe	His	Tyr	Asn	His	Ser	Lys	Val	Val	
		180					185					190				
GAG	TTT	CTG	GAA	GAC	AAG	GTT	GTC	TGC	TTT	GTG	TCC	TGG	TCC	TCG	GAT	801
Glu	Phe	Leu	Glu	Asp	Lys	Val	Val	Cys	Phe	Val	Ser	Trp	Ser	Ser	Asp	
		195				200						205				
CAC	CAC	CGC	CTC	ATC	TAC	ACC	ACC	TTT	CTG	CTG	CTC	TTC	CAA	TAC	TGC	849
His	His	Arg	Leu	Ile	Tyr	Thr	Thr	Phe	Leu	Leu	Leu	Phe	Gln	Tyr	Cys	
	210					215					220					
GTC	CCT	CTG	GCC	TTC	ATC	CTG	GTC	TGC	TAC	ATG	CGT	ATC	TAT	CAG	CGC	897
Val	Pro	Leu	Ala	Phe	Ile	Leu	Val	Cys	Tyr	Met	Arg	Ile	Tyr	Gln	Arg	
	225				230					235				240		
CTG	CAG	AGG	CAG	AGG	CGT	GCG	TTC	CAC	ACG	CAC	ACT	TGC	AGC	TCA	CGA	945
Leu	Gln	Arg	Gln	Arg	Arg	Ala	Phe	His	Thr	His	Thr	Cys	Ser	Ser	Arg	
			245						250					255		
GTG	GGG	CAG	ATG	AAG	CGG	ATC	AAT	GGC	ATG	CTC	ATG	GCA	ATG	GTG	ACT	993
Val	Gly	Gln	Met	Lys	Pro	Ile	Asn	Gly	Met	Leu	Met	Ala	Met	Val	Thr	
			260					265					270			
GCC	TTT	GCA	GTT	CTC	TGG	CTG	CCC	CTG	CAT	GTG	TTC	AAC	ACT	CTG	GAG	1041
Ala	Phe	Ala	Val	Leu	Trp	Leu	Pro	Leu	His	Val	Phe	Asn	Thr	Leu	Glu	
		275					280					285				
GAC	TGG	TAC	CAG	GAA	GCC	ATC	CCT	GCT	TGC	CAT	GGC	AAC	CTC	ATC	TTC	1089
Asp	Trp	Tyr	Gln	Glu	Ala	Ile	Pro	Ala	Cys	His	Gly	Asn	Leu	Ile	Phe	
	290					295					300					

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TTG ATG TGC CAC CTG TTT GCC ATG GCT TCC ACC TGT GTC AAC CCT TTC      1137
Leu Met Cys His Leu Phe Ala Met Ala Ser Thr Cys Val Asn Pro Phe
305                               310                               315                               320

ATC TAT GGC TTT CTC AAC ATC AAC TTC AAG AAG GAC ATC AAG GCT CTG      1185
Ile Tyr Gly Phe Leu Asn Ile Asn Phe Lys Lys Asp Ile Lys Ala Leu
                               325                               330                               335

GTT CTG ACC TGC CGT TGC AGG CCA CCT CAA GGG GAG CCT GAG CCT CTG      1233
Val Leu Thr Cys Arg Cys Arg Pro Pro Gln Gly Glu Pro Glu Pro Leu
                               340                               345                               350

CCC CTG TCC ACT GTG CAC ACG GAC CTC TCC AAG GGA TCT ATG AGG ATG      1281
Pro Leu Ser Thr Val His Thr Asp Leu Ser Lys Gly Ser Met Arg Met
                               355                               360                               365

GGT AGC AAG TCT AAC GTC ATG TAG T CATGTCTAGG CTCTTCCGCC      1326
Gly Ser Lys Ser Asn Val Met *
370                               375

ATTTCTTTTG ACACACCCTT TCACTGAGCT AAGTAGACAC AATGCAAGCT GTGGTATCAT      1386

CCTGCCATTT CTGGTCTTTG GGGCCCAGAC AGGCGGCAAG AGACTTGAAG CTT      1439

```

## (2) INFORMATION FOR SEQ ID NO:28:

## (i) SEQUENCE CHARACTERISTICS:

- (A) LENGTH: 376 amino acids
- (B) TYPE: amino acid
- (D) TOPOLOGY: linear

## (ii) MOLECULE TYPE: protein

## (xi) SEQUENCE DESCRIPTION: SEQ ID NO:28:

```

Met Asn Thr Ser His Leu Met Ala Ser Leu Ser Pro Ala Phe Leu Gln
 1                               5                               10                               15

Gly Lys Asn Gly Thr Asn Pro Leu Asp Ser Leu Tyr Asn Leu Ser Asp
                20                               25                               30

Gly Cys Gln Asp Ser Ala Asp Leu Leu Ala Phe Ile Ile Thr Thr Tyr
                35                               40                               45

Ser Val Glu Thr Val Leu Gly Val Leu Gly Asn Leu Cys Leu Ile Phe
 50                               55                               60

Val Thr Thr Arg Gln Lys Glu Lys Ser Asn Val Thr Asn Leu Leu Ile
 65                               70                               75                               80

Ala Asn Leu Ala Phe Ser Asp Phe Leu Met Cys Leu Ile Cys Gln Pro
                85                               90                               95

Leu Thr Val Thr Tyr Thr Ile Met Asp Tyr Trp Ile Phe Gly Glu Val
                100                               105                               110

Leu Cys Lys Met Leu Thr Phe Ile Gln Cys Met Ser Val Thr Val Ser
                115                               120                               125

Ile Leu Ser Leu Val Leu Val Ala Leu Glu Arg His Gln Leu Ile Ile
                130                               135                               140

Asn Pro Thr Gly Trp Lys Pro Ser Ile Ser Gln Ala Tyr Leu Gly Ile
145                               150                               155                               160

Val Val Ile Trp Phe Ile Ser Cys Phe Leu Ser Leu Pro Phe Leu Ala
                165                               170                               175

Asn Ser Ile Leu Asn Asp Leu Phe His Tyr Asn His Ser Lys Val Val

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What is claimed is:

1. An isolated nucleic acid molecule encoding a Y4  
5 receptor.
2. An isolated nucleic acid molecule of claim 1,  
wherein the nucleic acid molecule is a DNA  
10 molecule.
3. An isolated DNA molecule of claim 2, wherein the  
DNA molecule is a cDNA molecule.
4. An isolated DNA molecule of claim 2, wherein the  
15 DNA molecule is a genomic DNA molecule.
5. An isolated nucleic acid molecule of claim 1,  
wherein the nucleic acid molecule is an RNA  
20 molecule.
6. An isolated nucleic acid of claim 1 wherein the  
nucleic acid molecule encodes a human Y4  
receptor.
- 25 7. An isolated nucleic acid molecule of claim 6  
wherein the nucleic acid molecule encodes a  
receptor being characterized by an amino acid  
sequence in the transmembrane region, wherein  
the amino acid sequence has 60% homology or  
30 higher to the amino acid sequence in the  
transmembrane region of the human Y4 receptor  
shown in Figure 2.
8. An isolated nucleic acid molecule of claim 6  
35 wherein the human Y4 receptor has substantially  
the same amino acid sequence as shown in Figure  
1.

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9. An isolated nucleic acid molecule of claim 6 wherein the human Y4 receptor has the amino acid sequence as shown in Figure 1.
- 5 10. An isolated nucleic acid of claim 1 wherein the nucleic acid molecule encodes a rat Y4 receptor.
- 10 11. An isolated nucleic acid molecule of claim 10 wherein the rat Y4 receptor has substantially the same amino acid sequence as shown in Figure 3.
- 15 12. An isolated nucleic acid of claim 10 wherein the rat Y4 receptor has the amino acid sequence shown in Figure 3.
13. A purified Y4 receptor protein.
- 20 14. A vector comprising the nucleic acid molecule of claim 1.
15. A vector comprising the nucleic acid molecule of claim 6.
- 25 16. A vector comprising the nucleic acid molecule of claim 10.
- 30 17. A vector of claim 14 adapted for expression in a bacterial cell which comprises the regulatory elements necessary for expression of the nucleic acid in the bacterial cell operatively linked to the DNA encoding the Y4 receptor as to permit expression thereof.
- 35 18. A vector of claim 14 adapted for expression in a yeast cell which comprises the regulatory elements necessary for expression of the nucleic

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acid in the yeast cell operatively linked to the nucleic acid encoding the Y4 receptor as to permit expression thereof.

- 5      19. A vector of claim 14 adapted for expression in an insect cell which comprises the regulatory elements necessary for expression of the nucleic acid in the insect cell operatively linked to the nucleic acid encoding the Y4 receptor as to  
10      permit expression thereof.
20. A vector of claim 19 wherein the vector is a baculovirus.
- 15      21. A vector of claim 14 adapted for expression in a mammalian cell which comprises the regulatory elements necessary for expression of the nucleic acid in the mammalian cell operatively linked to the nucleic acid encoding the Y4 receptor as to  
20      permit expression thereof.
22. A vector of claim 15 adapted for expression in a mammalian cell which comprises the regulatory elements necessary for expression of the nucleic acid in the mammalian cell operatively linked to  
25      the nucleic acid encoding the Y4 receptor as to permit expression thereof.
23. A vector of claim 22 wherein the vector is a  
30      plasmid.
24. The plasmid of claim 23 designated pcEXV-Y4 (ATCC Accession No. 75631).
- 35      25. A vector of claim 16 adapted for expression in a mammalian cell which comprises the regulatory elements necessary for expression of the nucleic



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acid in the mammalian cell operatively linked to the nucleic acid encoding the Y4 receptor as to permit expression thereof.

- 5      26. A vector of claim 25 wherein the vector is a plasmid.
27. The plasmid of claim 26 designated pcEXV-rY4 (ATCC Accession No.      ).
- 10      28. A mammalian cell comprising the vector of claim 23 or 26.
29. A cell of claim 28 wherein the cell is non-neuronal in origin.
- 15      30. A cell of claim 28, wherein the cell is a COS-7 cell.
- 20      31. A cell of claim 27 wherein the cell is an LM(tk-) cell.
32. The cell of claim 31 designated L-hY4-3 (ATCC Accession No.      ).
- 25      33. A cell of claim 27 wherein the cell is an NIH-3T3 cell.
34. The cell of claim 33 designated N-hY4-5 (ATCC Accession No.      ).
- 30      35. A nucleic acid probe comprising a nucleic acid molecule of at least 15 nucleotides capable of specifically hybridizing with a unique sequence included within the sequence of a nucleic acid molecule encoding a Y4 receptor of claim 1.
- 35

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36. A nucleic acid probe of claim 35 wherein the nucleic acid encodes a human Y4 receptor.
- 5 37. A nucleic acid probe of claim 35 wherein the nucleic acid encodes a rat Y4 receptor.
38. The nucleic acid probe of claim 35 wherein the nucleic acid is DNA.
- 10 39. The nucleic acid probe of claim 35 wherein the nucleic acid is RNA.
- 15 40. An antisense oligonucleotide having a sequence capable of specifically hybridizing to an mRNA molecule encoding a Y4 receptor of claim 5 so as to prevent translation of the mRNA molecule.
- 20 41. An antisense oligonucleotide having a sequence capable of specifically hybridizing to the cDNA molecule of claim 3.
42. An antisense oligonucleotide of either of claims 40 or 41 comprising chemical analogues of nucleotides.
- 25 43. An antibody capable of binding to a Y4 receptor of claim 1.
- 30 44. An antibody of claim 43, wherein the Y4 receptor is a human Y4 receptor.
45. An antibody of claim 43, wherein the Y4 receptor is a rat Y4 receptor.
- 35 46. An antibody capable of competitively inhibiting the binding of the antibody of claim 43 to a Y4 receptor.

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47. An antibody of claim 43, wherein the antibody is a monoclonal antibody.
- 5 48. A monoclonal antibody of claim 47 directed to an epitope of a Y4 receptor present on the surface of a Y4 receptor expressing cell.
- 10 49. A pharmaceutical composition comprising an amount of the oligonucleotide of claim 40 effective to decrease activity of a Y4 receptor by passing through a cell membrane and binding specifically with mRNA encoding a Y4 receptor in the cell so as to prevent its translation and a pharmaceutically acceptable carrier capable of
- 15 passing through a cell membrane.
50. A pharmaceutical composition of claim 49, wherein the oligonucleotide is coupled to a substance which inactivates mRNA.
- 20 51. A pharmaceutical composition of claim 50, wherein the substance which inactivates mRNA is a ribozyme.
- 25 52. A pharmaceutical composition of claim 49, wherein the pharmaceutically acceptable carrier comprises a structure which binds to a receptor on a cell capable of being taken up by cells after binding to the structure.
- 30 53. A pharmaceutical composition of claim 52 wherein the structure of the pharmaceutically acceptable carrier is capable of binding to a receptor which is specific for a selected cell type.
- 35 54. A pharmaceutical composition which comprises an amount of the antibody of claim 43 effective to

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block binding of a ligand to a Y4 receptor and a pharmaceutically acceptable carrier.

- 5 55. A transgenic nonhuman mammal expressing nucleic acid encoding a Y4 receptor of claim 1.
- 10 56. A transgenic nonhuman mammal comprising a homologous recombination knockout of the native Y4 receptor.
- 15 57. A transgenic nonhuman mammal whose genome comprises antisense nucleic acid complementary to nucleic acid encoding a Y4 receptor of claim 1 so placed as to be transcribed into antisense mRNA which is complementary to mRNA encoding a Y4 receptor and which hybridizes to mRNA encoding a Y4 receptor thereby reducing its translation.
- 20 58. The transgenic nonhuman mammal of either of claims 55 or 57, wherein the nucleic acid encoding a Y4 receptor additionally comprises an inducible promotor.
- 25 59. The transgenic nonhuman mammal of either of claims 55 or 57, wherein the encoding a Y4 receptor additionally comprises tissue specific regulatory elements.
- 30 60. A transgenic nonhuman mammal of any of claims 55, 56 or 57, wherein the transgenic nonhuman mammal is a mouse.
- 35 61. A method for determining whether a ligand can specifically bind to a Y4 receptor which comprises contacting a cell transfected with and expressing nucleic acid encoding the Y4 receptor

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- of claim 1 with the ligand under conditions  
permitting binding of ligands to such receptor,  
and detecting the presence of any such ligand  
bound specifically to the Y4 receptor, thereby  
5 determining whether the ligand binds  
specifically to a Y4 receptor.
62. A method of claim 61 wherein the Y4 receptor is  
a human Y4 receptor.  
10
63. A method of claim 61 wherein the Y4 receptor is  
a rat Y4 receptor.
64. A method for determining whether a ligand can  
15 specifically bind to a Y4 receptor which  
comprises contacting a cell transfected with and  
expressing nucleic acid encoding the Y4 receptor  
of claim 1 with the ligand under conditions  
permitting binding of ligands to such receptor,  
20 and detecting the presence of any such ligand  
bound specifically to the Y4 receptor, thereby  
determining whether the ligand binds  
specifically to a Y4 receptor, wherein the Y4  
receptor is characterized by an amino acid  
25 sequence in the transmembrane region, such amino  
acid sequence having 60% homology or higher to  
the amino acid sequence in the transmembrane  
region of the human Y4 receptor shown in Figure  
2.
- 30
65. A method of claim 64 wherein the Y4 receptor is  
a human Y4 receptor.
66. A method of claim 64 wherein the Y4 receptor is  
35 a rat Y4 receptor.
67. A method for determining whether a ligand can

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bind specifically to a Y4 receptor which  
comprises preparing a cell extract from cells  
transfected with and expressing nucleic acid  
encoding the Y4 receptor of claim 1, isolating a  
5 membrane fraction from the cell extract,  
contacting the ligand with the membrane fraction  
under conditions permitting binding of ligands  
to such receptor, and detecting the presence of  
any ligand bound to the Y4 receptor, thereby  
10 determining whether the compound is capable of  
specifically binding to a Y4 receptor.

68. A method of claim 67 wherein the Y4 receptor is  
a human Y4 receptor.

15 69. A method of claim 67 wherein the Y4 receptor is  
a rat Y4 receptor.

20 70. A method of any of claims 61, 62, 63, 64, 65,  
66, 67, 68, or 69 wherein the ligand is not  
previously known.

71. A ligand determined by the method of claim 70.

25 72. A method for determining whether a ligand is a  
Y4 receptor agonist which comprises contacting a  
cell transfected with and expressing nucleic  
acid encoding a Y4 receptor with the ligand  
under conditions permitting the activation of a  
30 functional Y4 receptor response from the cell,  
and detecting by means of a bioassay, such as a  
second messenger response, an increase in Y4  
receptor activity, thereby determining whether  
the ligand is a Y4 receptor agonist.

35 73. A method for determining whether a ligand is a  
Y4 receptor agonist which comprises preparing a

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- 5 cell extract from cells transfected with and  
expressing nucleic acid encoding a Y4 receptor  
of claim 1, isolating a membrane fraction from  
the cell extract, contacting the membrane  
fraction with the ligand under conditions  
10 permitting the activation of a functional Y4  
receptor response and detecting by means of a  
bioassay, such as a second messenger response,  
an increase in Y4 receptor activity, thereby  
determining whether the ligand is a Y4 receptor  
agonist.
- 15 74. A method of either of claims 72 or 73 wherein  
the Y4 receptor is a human Y4 receptor.
75. A method of either of claims 72 or 73 wherein  
the Y4 receptor is a rat Y4 receptor.
- 20 76. A method for determining whether a ligand is a  
Y4 receptor antagonist which comprises  
contacting a cell transfected with and  
expressing nucleic acid encoding a Y4 receptor  
of claim 1 with the ligand in the presence of a  
known Y4 receptor agonist, such as PP, under  
25 conditions permitting the activation of a  
functional Y4 receptor response and detecting by  
means of a bioassay, such as a second messenger  
response, a decrease in Y4 receptor activity,  
thereby determining whether the ligand is a Y4  
30 receptor antagonist.
- 35 77. A method for determining whether a ligand is a  
Y4 receptor antagonist which comprises preparing  
a cell extract from cells transfected with and  
expressing nucleic acid encoding a Y4 receptor  
of claim 1, isolating a membrane fraction from  
the cell extract, contacting the membrane

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- fraction of the extract with the ligand in the presence of a known Y4 receptor agonist, such as PP, under conditions permitting the activation of a functional Y4 receptor response and  
5 detecting by means of a bioassay, such as a second messenger response, a decrease in Y4 receptor activity, thereby determining whether the ligand is a Y4 receptor antagonist.
- 10 78. A method of either of claims 76 or 77 wherein the Y4 receptor is a human Y4 receptor.
79. A method of either of claims 76 or 77 wherein the Y4 receptor is a rat Y4 receptor.
- 15 80. A method of any of claims 72, 73, 76 or 77 wherein the second messenger assay comprises measurement of intracellular cAMP.
- 20 81. A method of any of claims 72, 73, 76, or 77 wherein the second messenger assay comprises measurement of intracellular calcium mobilization.
- 25 82. A method of any of claims 61, 62, 63, 64, 65, 66, 67, 68, 69, 70, 71, 72, 73, 74, 75, 76, 77, 78 or 79 wherein the cell is a mammalian cell.
- 30 83. The method of claim 82 wherein the mammalian cell is nonneuronal in origin.
84. A method of claim 83, wherein the mammalian cell is nonneuronal in origin is a COS-7 cell.
- 35 85. A method of claim 83, wherein the mammalian cell nonneuronal in origin is a CHO cell.



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86. A method of claim 83, wherein the mammalian cell nonneuronal in origin is a LM(tk-) cell.
87. A method of claim 83, wherein the mammalian cell nonneuronal in origin is a NIH-3T3 cell.
88. A ligand detected by the method of any of claims 61, 62, 63, 64, 65, 66, 67, 68, 69, 70, 71, 72, 73, 74, 75, 76, 77, 78, 79, 80 or 81.
89. A ligand of claim 88 wherein the ligand is not previously known.
90. A pharmaceutical composition which comprises an amount of a Y4 receptor agonist determined by the method of either of claims 72 or 73 effective to reduce activity of a Y4 receptor and a pharmaceutically acceptable carrier.
91. A pharmaceutical composition of claim 90 wherein the Y4 receptor agonist is not previously known.
92. A pharmaceutical composition which comprises an amount of a Y4 receptor antagonist determined by the method of either of claims 76 or 77 effective to increase activity of Y4 receptor and a pharmaceutically acceptable carrier.
93. A pharmaceutical composition of claim 92 wherein the Y4 receptor antagonist is not previously known.
94. A method of screening drugs to identify drugs which specifically bind to a Y4 receptor on the surface of a cell which comprises contacting a cell transfected with and expressing nucleic acid encoding a Y4 receptor with a plurality of

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5 drugs under conditions permitting binding of drugs to the Y4 receptor, and determining those drugs which specifically bind to the transfected cell, thereby identifying drugs which specifically bind to a Y4 receptor.

10 95. A method of screening drugs to identify drugs which specifically bind to a Y4 receptor on the surface of a cell which comprises preparing a cell extract from cells transfected with and expressing nucleic acid encoding a Y4 receptor, isolating a membrane fraction from the cell extract, contacting the membrane fraction with a plurality of drugs, and determining those drugs  
15 which bind to the transfected cell, thereby identifying drugs which specifically bind to a Y4 receptor.

20 96. A method of either of claims 94 or 95 wherein the Y4 receptor is a human Y4 receptor.

97. A method of either of claims 94 or 95 wherein the Y4 receptor is a rat Y4 receptor.

25 98. A method of screening drugs to identify drugs which act as agonists of Y4 receptor which comprises contacting a cell transfected with and expressing nucleic acid encoding a Y4 receptor with a plurality of drugs under conditions  
30 permitting the activation of a functional Y4 receptor response, and determining those drugs which activate such receptor using a bioassay such, as a second messenger assay, thereby identifying drugs which act as Y4 receptor  
35 agonists.

99. A method of screening drugs to identify drugs

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5 which act as agonists of Y4 receptor which  
comprises preparing a cell extract from cells  
transfected with and expressing nucleic acid  
encoding a Y4 receptor, isolating a membrane  
fraction from the cell extract, contacting the  
membrane fraction with a plurality of drugs  
under conditions permitting the activation of a  
functional Y4 receptor response, and determining  
those drugs which activate such receptor using a  
10 bioassay, such as a second messenger assay,  
thereby identifying drugs which act as Y4  
receptor agonists.

15 100. A method of either of claims 98 or 99 wherein  
the Y4 receptor is a human Y4 receptor.

101. A method of either of claims 98 or 99 wherein  
the Y4 receptor is a rat Y4 receptor.

20 102. A method of screening drugs to identify drugs  
which act as Y4 receptor antagonists which  
comprises contacting a cell transfected with and  
expressing nucleic acid encoding a Y4 receptor  
with a plurality of drugs in the presence of a  
25 known Y4 receptor agonist, such as PP, under  
conditions permitting the activation of a  
functional Y4 receptor response, and determining  
those drugs which inhibit the activation of the  
receptor using a bioassay, such as a second  
30 messenger assay, thereby identifying drugs which  
act as Y4 receptor antagonists.

103. A method of screening drugs to identify drugs  
which act as Y4 receptor antagonists which  
35 comprises preparing a cell extract from cells  
transfected with and expressing nucleic acid  
encoding a Y4 receptor, isolating a membrane

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fraction from the cell extract, contacting the membrane fraction with a plurality of drugs in the presence of a known Y4 receptor agonist, such as PP, under conditions permitting the activation of a functional Y4 receptor response, and determining those drugs which inhibit the activation of the receptor using a bioassay, such as a second messenger assay, thereby identifying drugs which act as Y4 receptor antagonists.

104. A method of either of claims 102 or 103 wherein the Y4 receptor is a human Y4 receptor.
105. A method of either of claims 102 or 103 wherein the Y4 receptor is a rat Y4 receptor.
106. A method of any of claims 98, 99, 102 or 103 wherein the second messenger assay comprises measurement of intracellular cAMP.
107. A method of any of claims 98, 99, 102 or 103 wherein the second messenger assay comprises measurement of intracellular calcium mobilization.
108. The method of any of claims 94, 95, 96, 97, 98, 99, 100, 101, 102, 103, 104, or 105 wherein the cell is a mammalian cell.
109. The method of claim 108 wherein the mammalian cell is nonneuronal in origin.
110. The method of claim 109 wherein the mammalian cell nonneuronal in origin is a Cos-7 cell.
111. The method of claim 109 wherein the mammalian

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cell nonneuronal in origin is a CHO cell.

112. The method of claim 109 wherein the mammalian cell nonneuronal in origin is a LM(tk-) cell.

5

113. The method of claim 109 wherein the mammalian cell nonneuronal in origin is an NIH-3T3 cell.

10

114. A pharmaceutical composition comprising a drug identified by the method of either of claims 98 or 99 and a pharmaceutically acceptable carrier.

15

115. A pharmaceutical composition comprising a drug identified by the method of either of claims 102 or 103 and a pharmaceutically acceptable carrier.

20

116. A method of detecting expression of a Y4 receptor by detecting the presence of mRNA coding for a Y4 receptor which comprises obtaining total mRNA from the cell and contacting the mRNA so obtained with the nucleic acid probe of claim 40 under hybridizing conditions, and detecting the presence of mRNA hybridized to the probe, thereby detecting the expression of a Y4 receptor by the cell.

25

117. A method of treating an abnormality in a subject, wherein the abnormality is alleviated by decreasing the activity of a Y4 receptor which comprises administering to a subject an effective amount of the pharmaceutical composition of either of claims 90 or 114, thereby treating the abnormality.

30

118. A method of treating an abnormality in a subject wherein the abnormality is alleviated by

35

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5 decreasing the activity of Y4 receptor which comprises administering to a subject an effective amount of a Y4 receptor antagonist determined by the methods of any of claims 76, 77, 102, or 103, thereby treating the abnormality.

10 119. The method of either of claims 117 or 118 wherein the abnormal condition is amnesia.

120. The method of either of claims 117 or 118 wherein the abnormal condition is a feeding disorder.

15 121. The method of either of claims 117 or 118 wherein the abnormal condition is epilepsy.

20 122. The method of either of claims 117 or 118 wherein the abnormal condition is hypertension.

123. The method of either of claims 117 or 118 wherein the abnormal condition is sleeping disorder.

25 124. The method of either of claims 117 or 118 wherein the abnormal condition is pain.

30 125. A method of treating an abnormality in a subject, wherein the abnormality is alleviated by decreasing the activity of a human Y4 receptor which comprises administering to the subject an amount of the pharmaceutical composition of claim 54 effective to block binding of ligands to a Y4 receptor, thereby  
35 treating the abnormality.

126. A method of treating an abnormality in a

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subject, wherein the abnormality is alleviated  
by decreasing the activity of a human Y4  
receptor which comprises administering to the  
subject an effective amount of the  
5 pharmaceutical composition of claim 49, thereby  
treating the abnormality.

127. The method of either of claims 125 or 126  
wherein the abnormal condition is amnesia.

128. The method of either of claims 125 or 126  
wherein the abnormal condition is a feeding  
disorder.

129. The method of either of claims 125 or 126  
wherein the abnormal condition is epilepsy.

130. The method of either of claims 125 or 126  
wherein the abnormal condition is hypertension.

131. The method of either of claims 125 or 126  
wherein the abnormal condition is sleeping  
disorder.

132. The method of either of claims 125 or 126  
wherein the abnormal condition is pain.

133. A method of detecting the presence of a Y4  
receptor on the surface of a cell which  
comprises contacting the cell with the antibody  
of claim 43 under conditions permitting binding  
of the antibody to the receptor, and detecting  
the presence of the antibody bound to the cell,  
thereby detecting the presence of a Y4 receptor  
on the surface of the cell.

134. A method of determining the physiological

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- 5 effects of expressing varying levels of Y4  
receptors which comprises producing a transgenic  
nonhuman mammal of claim 55 whose levels of Y4  
receptor expression are varied by use of an  
inducible promoter which regulates Y4 receptor  
expression.
- 10 135. A method of determining the physiological  
effects of expressing varying levels of Y4  
receptors which comprises producing a panel of  
transgenic nonhuman mammals of claim 55 each  
expressing a different amount of Y4 receptor.
- 15 136. A method for identifying a Y4 receptor  
antagonist capable of alleviating an abnormality  
in a subject, wherein the abnormality is  
alleviated by decreasing the activity of a Y4  
receptor which comprises administering the  
antagonist to a transgenic nonhuman mammal of  
20 any of claims 55, 57, 58, 59 or 60 and  
determining whether the antagonist alleviates  
the physical and behavioral abnormalities  
displayed by the transgenic nonhuman mammal as a  
result of activity of a Y4 receptor, thereby  
25 identifying a Y4 antagonist.
137. An antagonist identified by the method of claim  
136.
- 30 138. A pharmaceutical composition comprising an  
antagonist identified by the method of claim 136  
and a pharmaceutically acceptable carrier.
- 35 139. A method of treating an abnormality in a subject  
wherein the abnormality is alleviated by  
decreasing the activity of a Y4 receptor which  
comprises administering to the subject an



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effective amount of the pharmaceutical composition of claim 138, thereby treating the abnormality.

5      140. A method for identifying a Y4 receptor agonist capable of alleviating an abnormality in a subject wherein the abnormality is alleviated by activation of a Y4 receptor which comprises administering the agonist to the transgenic  
10      nonhuman mammal of any of claims 55, 56, 57, 58, 59 or 60 and determining whether the substance alleviates the physical and behavioral abnormalities displayed by the transgenic nonhuman mammal, the alleviation of the  
15      abnormality indicating the identification of a Y4 receptor agonist.

141. An agonist identified by the method of claim 140.

20

142. A pharmaceutical composition comprising an agonist identified by the method of claim 140 and a pharmaceutically acceptable carrier.

25      143. A method for treating an abnormality in a subject wherein the abnormality is alleviated by activation of a Y4 receptor which comprises administering to the subject an effective amount of the pharmaceutical composition of claim 142,  
30      thereby treating the abnormality.

144. A method for diagnosing a predisposition to a disorder associated with the activity of a specific Y4 receptor allele which comprises:

35

a. obtaining DNA of subjects suffering from the disorder;

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- b. performing a restriction digest of the DNA with a panel of restriction enzymes;
- 5 c. electrophoretically separating the resulting DNA fragments on a sizing gel;
- d. contacting the resulting gel with a nucleic acid probe capable of specifically hybridizing to DNA encoding a Y4 receptor and labelled with a detectable marker;
- 10 e. detecting labelled bands which have hybridized to the DNA encoding a Y4 receptor labelled with a detectable marker to create a unique band pattern specific to the DNA of subjects suffering from the disorder;
- 15 f. preparing DNA obtained for diagnosis by steps a-e; and
- 20 g. comparing the unique band pattern specific to the DNA of subjects suffering from the disorder from step e and the DNA obtained for diagnosis from step f to determine whether the patterns are the same or different and to diagnose thereby predisposition to the disorder if the patterns are the same.
- 25
- 30
145. The method of claim 144 wherein a disorder associated with the expression of a specific human Y4 receptor allele is diagnosed.
- 35 146. A method of preparing the purified isolated Y4 receptor of claim 13 which comprises:

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- 5 a. constructing a vector adapted for  
expression in a cell which comprises the  
regulatory elements necessary for the  
expression of nucleic acid in the cell  
operatively linked to the nucleic acid  
encoding a Y4 receptor as to permit  
expression thereof, wherein the cell is  
selected from the group consisting of  
bacterial cells, yeast cells, insect cells  
10 and mammalian cells;
- b. inserting the vector of step a in a  
suitable host cell;
- 15 c. incubating the cells of step b under  
conditions allowing the expression of a Y4  
receptor;
- 20 d. recovering the receptor so produced; and
- e. purifying the receptor so recovered,  
thereby preparing an isolated Y4 receptor.

FIGURE 1-1

FIGURE 1-1
FIGURE 1-2
FIGURE 1-3
FIGURE 1-4
FIGURE 1-5

-28	AGTATTGTTTGTCTGTTTGCCTTGTAGGGCGTCATCCCTCAAGTGATCACTTAGTTCAA	31
32	GAGTCCCTGGAATCTTTTCACATCCACTATGAACACCTCTCACCTCCTGGCCTTGCTGCTC	91
-8	M N T S H L L A L L L	11
92	CCAAAATCTCCACAAGGTGAAAAACAGAAACCCCTGGGCACCCCATACAACTTCTCT	151
12	P K S P Q G E N R S K P L G T P Y N F S	31
152	GAACATTGCCAGGATTCCCGTGGACGTGATGGTCTTCATCGTCACTTCCTACAGCATTGAG	211
32	E H C Q D S V D V M V F I V T S Y S I E	51
212	ACTGTCGTGGGGTCCCTGGGTAAACCTCTGCCTGATGTGTGACTGTGAGGCAGAAGGAG	271
52	T V V G V L G N L C L M C V T V R Q K E	71

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FIGURE 1-2

220	240	260	
272	AAAGCCAAACGTGACCAACCTGCTTATCGCCCAACCTGGCCCTTCTCTGACTTCCTCATGTGC	331	
72	K A N V T N L L I A N L A F S D F L M C	91	
280	300	320	
332	CTCCTCTGCCAGCCGCTGACCGCGTCTACACCATCATGGACTACTGGATCTTTGGAGAG	391	
92	L L C Q P L T A V Y T I M D Y W I F G E	111	
340	360	380	
392	ACCCTCTGCAAGATGTCGGCCCTTCATCCAGTGCATGTCGGTGACGGTCTCCATCCTCTCG	451	
112	T L C K M S A F I Q C M S V T V S I L S	131	
400	420	440	
452	CTCGTCCTCGTGGCCCTGGAGAGGCATCAGCTCATCATCAACCAACAGGCTGGAAGCCC	511	
132	L V L V A L E R H Q L I I N P T G W K P	151	
460	480	500	
512	AGCATCTCACAGGCCCTACCTGGGGATTGTGCTCATCTGGGTCAATTGCCCTGTGTCCTCTCC	571	
152	S I S Q A Y L G I V L I W V I A C V L S	171	

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FIGURE 1-3

520	540	560	
572 C T G C C C T T C C T G G C C A A C A G C A T C C T G G A G A T G T C T T C C A C A G A A C C A C T C C A A G G C T			631
172 L P F L A N S I L E N V F H K N H S K A			191
580	600	620	
632 C T G G A G T T C C T G G C A G A T A A G G T G T C T G T A C C G A G T C C T G G C C A C T G G C T C A C C A C C G C			691
192 L E F L A D K V V C T E S W P L A H H R			211
640	660	680	
692 A C C A T C T A C A C C A C C T T C C T G C T C C T C T T C C A G T A C T G C C T C C C A C T G G G C T T C A T C C T G			751
212 T I Y T T F L L L L F Q Y C L P L G F I L			231
700	720	740	
752 G T C T G T T A T G C A C G C A T C T A C C G G C C T G C A G A G G C A G G G C G T G T T C A C A A G G G C			811
232 V C Y A R I Y R R L Q R Q R Q G R V F H K G			251
760	780	800	
812 A C C T A C A G C T T G C G A G C T G G C A C A T G A A G C A G G T C A A T G T G T G C T G G T G G T G A T G G T G			871
252 T Y S L R A G H M K Q V N V V L V V M V			271

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FIGURE 1-4

872	GTGGCCTTTGCGCTCTGGCTGCCCTCTGCATGTGTCAACAGCCTGGAAGACTGGCAC	820	840	860	931
272	V A F A V L W L P L H V F N S L E D W H				291
		880	900	920	
932	CATGAGGCCATCCCCATCTGCCACGGGAACCTCATCTTCTTAGTGTGCCACTTGCTTGCC				991
292	H E A I P I C H G N L I F L V C H L L A				311
		940	960	980	
992	ATGGCCTCCACCTGCGTCAACCCATTCATCTATGGCTTCTCAACACCAACTTCAAGAAG				1051
312	M A S T C V N P F I Y G F L N T N F K K				331
		1000	1020	1040	
1052	GAGATCAAGGCCCTGGTGCTGACTTGCCAGCAGAGCGCCCCCTGGAGGAGTCGGAGCAT				1111
332	E I K A L V L T C Q Q S A P L E E S E H				351
		1060	1080	1100	
1112	CTGCCCTGTCCACAGTACATACGGAAGTCTCCAAGGGTCCCTGAGGCTAAGTGGCAGG				1171
352	L P L S T V H T E V S K G S L R L S G R				371

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## FIGURE 1-5

1172	1120	1140	1160	1231
372	TCCAATCCCAATTTAAACCAGGTCTAGGTCTTCTCCCTGCCATGTCCCTTGCCAGGCTCTTC			375
	S N P I *			
1232	1180	1200	1220	1291
	CACTTAGCTAAGTGGGCACACTGCAAGCTGGGGTGGCACCCAGCAATTCCTGGCTTCTG			



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FIGURE 2-1  
FIGURE 2-2  
FIGURE 2-3

FIGURE 2-1

1		50
hp25a	MNTSHLLALL LPKSPQGENR SKPLGTPYNF SEHCQDSVDV MVFIVTSYSI	
human Y1	MN.STLFSQV ENHSVHSNFS EKNAQLLAFE NDDCHLPLAM IFTLALAYGA	
rat Y1	MN.STLFSRV ENYSVHYNVS E.NSPFLAFE NDDCHLPLAV IFTLALAYGA	
mouse Y1	MN.STLFSKV ENHSIHYNAS E.NSPLLAFE NDDCHLPLAV IFTLALAYGA	
51		100
hp25a	ETVVGVLGNL CLMCVTVRQK	
human Y1	VIILGVSGNL ALIIIIILKQK	
rat Y1	VIILGVSGNL ALIIIIILKQK	
mouse Y1	VIILGVSGNL ALIIIIILKQK	
101		150
hp25a	YTIMDYWIFG ETLCKMSAFI QCMSVTVSIL SLVLVALERH QLIINPTGWK	
human Y1	YTLMDHWVFG EAMCKLNPFV QCVSITVSIF SLVLI AVERH QLIINPRGWR	
rat Y1	YTLMDHWVFG ETLCKLNPFV QCVSITVSIF SLVLI AVERH QLIINPRGWR	
mouse Y1	YTLMDHWVFG ETLCKLNPFV QCVSITVSIF SLVLI AVERH QLIINPRGWR	
151		200
hp25a	PSISQAYIGI VLIWVIAVCVL SLPFLANSIL	
human Y1	PNNRHAYVGI AVIWVLAVAS SLPFLIYQVM TDEPFQNVV	
rat Y1	PNNRHAYIGI TVIWVLAVAS SLPFLIYQIL TDEPFQNVV	
mouse Y1	PNNRHAYIGI TVIWVLAVAS SLPFLIYQIL TDEPFQNVV	

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FIGURE 2-2

hp25a	201	CTESWPLAHH	RTIYTTFLL	FQYCLPLGFI	LVCYARIYRR	LQRQGRVFBK	250
human Y1		CFDQFPDSH	RLSYTTLLV	LQYFGPLCFI	FICYFKIYIR	LKRRNNMMDK	
rat Y1		CFDKFPDSH	RLSYTTLLV	LQYFGPLCFI	FICYFKIYIR	LKRRNNMMDK	
mouse Y1		CFDKFPDSH	RLSYTTLLV	LQYFGPLCFI	FICYFKIYIR	LKRRNNMMDK	
			V				
	251						300
hp25a		GTYS.LRAGH	MKQNVNVLV	MVAFAVLWL	PLHVFNSLD	WHHEAIPICH	
human Y1		MRDNKYRSSE	TKRINIMLLS	IVAFAVCWL	PLTIFNTVFD	WNHQIIATCN	
rat Y1		IRDSKYRSSE	TKRINVMLLS	IVAFAVCWL	PLTIFNTVFD	WNHQIIATCN	
mouse Y1		IRDSKYRSSE	TKRINIMLLS	IVAFAVCWL	PLTIFNTVFD	WNHQIIATCN	
			VI				
	301						350
hp25a		GNIIFLVCHL	LAMASTCVNP	FIYGFINTNF	KKEIKALVLT	CQQSAPLEES	
human Y1		HNLLFLLCHL	TAMISTCVNP	IFYGFINKNF	ORDLQFFNF	CDFRSRDDY	
rat Y1		HNLLFLLCHL	TAMISTCVNP	IFYGFINKNF	ORDLQFFNF	CDFRSRDDY	
mouse Y1		HNLLFLLCHL	TAMISTCVNP	IFYGFINKNF	ORDLQFFNF	CDFRSRDDY	
			VII				

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FIGURE 2-3

hp25a	351	388
human Y1	EHLPLSTVHT	EVSKGSLRLS
rat Y1	ETIAMSTMHT	DVSKTSLKQA
mouse Y1	ETIAMSTMHT	DVSKTSLKQA
	ETIAMSTMHT	DVSKTSLKQA
		GRSNPI*
		SPVAFKKINN
		SPVAFKKISM
		N.DNEKI*
		N.DNEKI*
		N.DNEKV*

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## FIGURE 3-1

FIGURE 3-1
FIGURE 3-2
FIGURE 3-3
FIGURE 3-4

-170                      -150                      -130

ATAGCTCTCAAGCCATAAGATATAAGTAGCTAAGAATTGTCTCCCTCTCCCTGTCCCTTG

-110                      -90                      -70

TTCTTACCTGGTTCCATTTTACATGCCTGGACCTTTGAGTTCCATTTGTTTGTTCAG

-50                      -30                      -10

GCTACACTCAGAAGTGGGCCCTTTAGTCTTGAAGTTCCTGGTCTTCTCACACCCACCATG

M

10                      30                      50

AATACCTCTCATCTCATGGCCTCCCTTTCTCCGGCATTCTTACAAGGTAAGAATGGGACC

N T S H L M A S L S P A F L Q G K N G T

70                      90                      110

AACCCACTGGATTCCCTCTATAATCTCTCTGACGGCTGCCAGGATTCGGCAGATCTGTTG

N P L D S L Y N L S D G C Q D S A D L L

130                      150                      170

GCCTTCATCATCACCACCTACAGCGTTGAGACCGTCTTGGGGGTCCTAGGAAACCTCTGC

A F I I T T Y S V E T V L G V L G N L C

190                      210                      230

TTGATATTTGTGACCACAAGGCAAAAGGAAAAGTCCAATGTGACCAACCTACTCATTGCC

L I F V T T R Q K E K S N V T N L L I A

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## FIGURE 3-2

250 270 290  
AACCTGGCCTTCTCTGACTTCCTCATGTGTCTCATCTGCCAGCCGCTCACGGTCACCTAC  
N L A F S D F L M C L I C Q P L T V T Y

310 330 350  
ACCATCATGGACTACTGGATCTTCGGCGAAGTCCTTTGCAAGATGTTAACGTTTCATCCAG  
T I M D Y W I F G E V L C K M L T F I Q

370 390 410  
TGTATGTCGGTGACAGTCTCCATCCTCTCACTGGTCCTTGTGGCCCTGGAGAGGCACCAG  
C M S V T V S I L S L V L V A L E R H Q

430 450 470  
CTCATTATCAACCCGACTGGCTGGAAACCCAGCATTTCCCAGGCCTACCTGGGGATTGTG  
L I I N P T G W K P S I S Q A Y L G I V

490 510 530  
GTCATCTGGTTCATTTCTTGTTTCCTCTCCTTGCCCTTCCTGGCCAATAGCATCCTGAAC  
V I W F I S C F L S L P F L A N S I L N

550 570 590  
GACCTCTTCCACTACAACCACTCTAAGGTTGTGGAGTTTCTGGAAGACAAGGTTGTCTGC  
D L F H Y N H S K V V E F L E D K V V C

610 630 650  
TTTGTGTCCTGGTCCTCGGATCACCACCGCCTCATCTACACCACCTTTCTGCTGCTCTTC  
F V S W S S D H H R L I Y T T F L L L F

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## FIGURE 3-3

670 690 710  
CAATACTGCGTCCCTCTGGCCTTCATCCTGGTCTGCTACATGCGTATCTATCAGCGCCTG  
Q Y C V P L A F I L V C Y M R I Y Q R L

730 750 770  
CAGAGGCAGAGGCGTTCGTTCCACACGCACACTTGCAGCTCACGAGTGGGGCAGATGAAG  
Q R Q R R A F H T H T C S S R V G Q M K

790 810 830  
CGGATCAATGGCATGCTCATGGCAATGGTGA CTGCCTTTGCAGTTCTCTGGCTGCCCCCTG  
R I N G M L M A M V T A F A V L W L P L

850 870 890  
CATGTGTTCAACACTCTGGAGGACTGGTACCAGGAAGCCATCCCTGCTTGCCATGGCAAC  
H V F N T L E D W Y Q E A I P A C H G N

910 930 950  
CTCATCTTCTTGATGTGCCACCTGTTTGCCATGGCTTCCACCTGTGTCAACCCTTTTCATC  
L I F L M C H L F A M A S T C V N P F I

970 990 1010  
TATGGCTTTCTCAACATCAACTTCAAGAAGGACATCAAGGCTCTGGTTCTGACCTGCCGT  
Y G F L N I N F K K D I K A L V L T C R

1030 1050 1070  
TGCAGGCCACCTCAAGGGGAGCCTGAGCCTCTGCCCCTGTCCACTGTGCACACGGACCTC  
C R P P Q G E P E P L P L S T V H T D L

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## FIGURE 3-4

1090 1110 1130  
TCCAAGGGATCTATGAGGATGGGTAGCAAGTCTAACGTCATGTAGTCATGTCTAGGCTCT  
S K G S M R M G S K S N V M \*

1150 1170 1190  
TCCGCCATTTTCTTTCGACACACCCTTTCCTGAGCTAAGTAGACACAATGCAAGCTGTG

1210 1230 1250  
GTATCATCCTGCCATTTCTGGTCTTTGGGGCCCAGACAGGCGGCAAGAGACTTGAAGCTT

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## FIGURE 4

1 50

Y4rat MNTSHLMASL SPAFLQGKNG TNPLDSLYNL SDGCQDSADL LAFIITTSV  
Y4hum MNTSHLLALL LPKSPQGENR SKPLGTPYNF SEHCQDSVDV MVFIVTSYSI

51 100

— I — II —

Y4rat ETVLGVLGNL CLIFVTTRQK EKSNTNLLI ANLAFSDFLM CLICQPLTVT  
Y4hum ETVVGVLGNL CLMCVTVRQK EKANVTNLLI ANLAFSDFLM CLLCQPLTAV

101 150

— III —

Y4rat YTIMDYWIFG EVLCKMLTFI QCMSVTVSIL SLVLVALERH QLIINPTGWK  
Y4hum YTIMDYWIFG ETLCKMSAPI QCMSVTVSIL SLVLVALERH QLIINPTGWK

151 200

— IV —

Y4rat PSISQAYLGI VVIWFISCFL SLPFLANSIL NDLFHYNHNSK VVEFLEDKVV  
Y4hum PSISQAYLGI VLIWVIACVL SLPFLANSIL ENVFHKHNSK ALEFLADKVV

201 250

— V —

Y4rat CFVSWSSDHH RLIYTTFLLL PQYCVPLAFI LVCYMRIYQR LQRORRAFHT  
Y4hum CTESWPLAHH RTIYTTFLLL PQYCLPLGFI LVCYARIYRR LQRQGRVPHK

251 300

— VI —

Y4rat HTCSSRVGQM KRINGMLMAM VTAPAVLWLP LHVFNLTLEDW YQEAIPACHG  
Y4hum GTYSLRAGHM KQNVVVLVVM VVAPAVLWLP LHVFNLSLEDW HHEAIPICHG

301 350

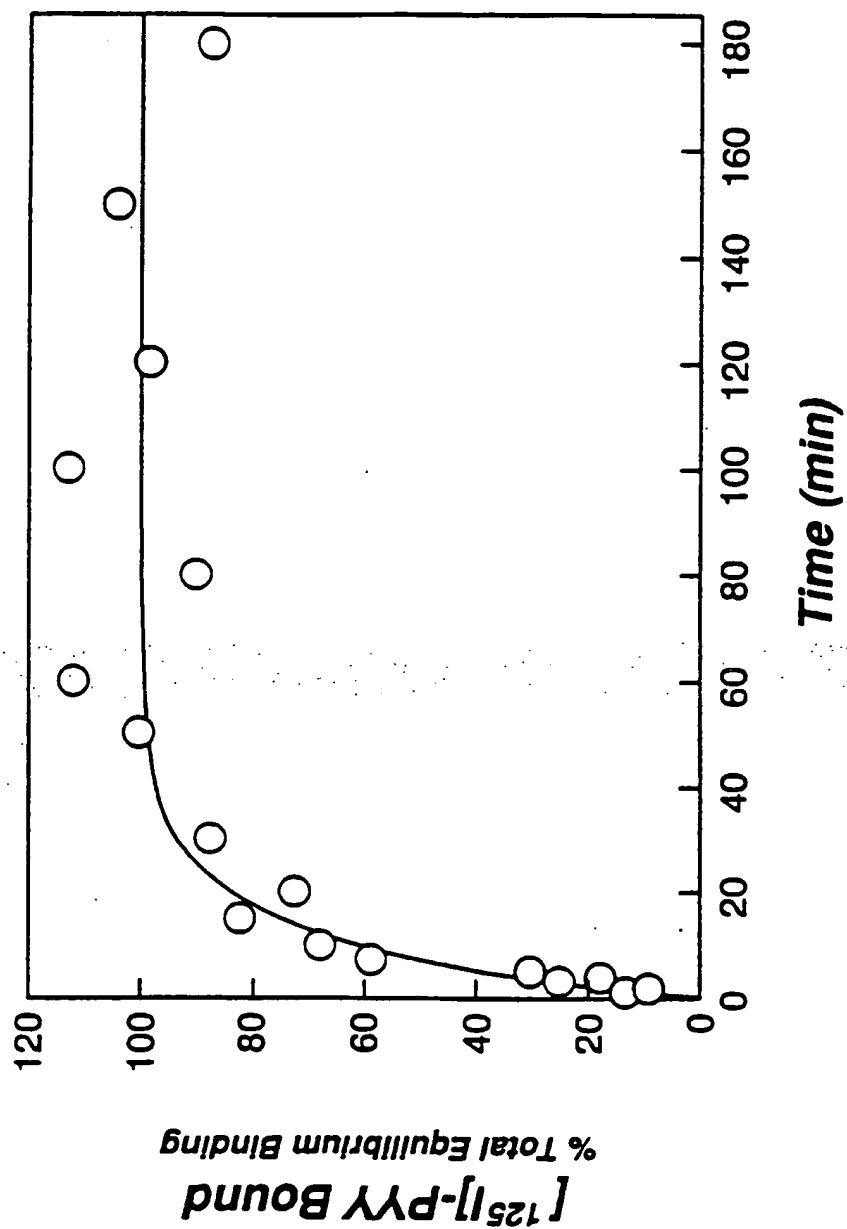
— VII —

Y4rat NLIFLMCHLP AMASTCVNPF IYGFLNINFK KDIKALVLTG RCRPPQGEPE  
Y4hum NLIFLVCHLL AMASTCVNPF IYGFLNTNFK KEIKALVLTG QQSAPLEESE

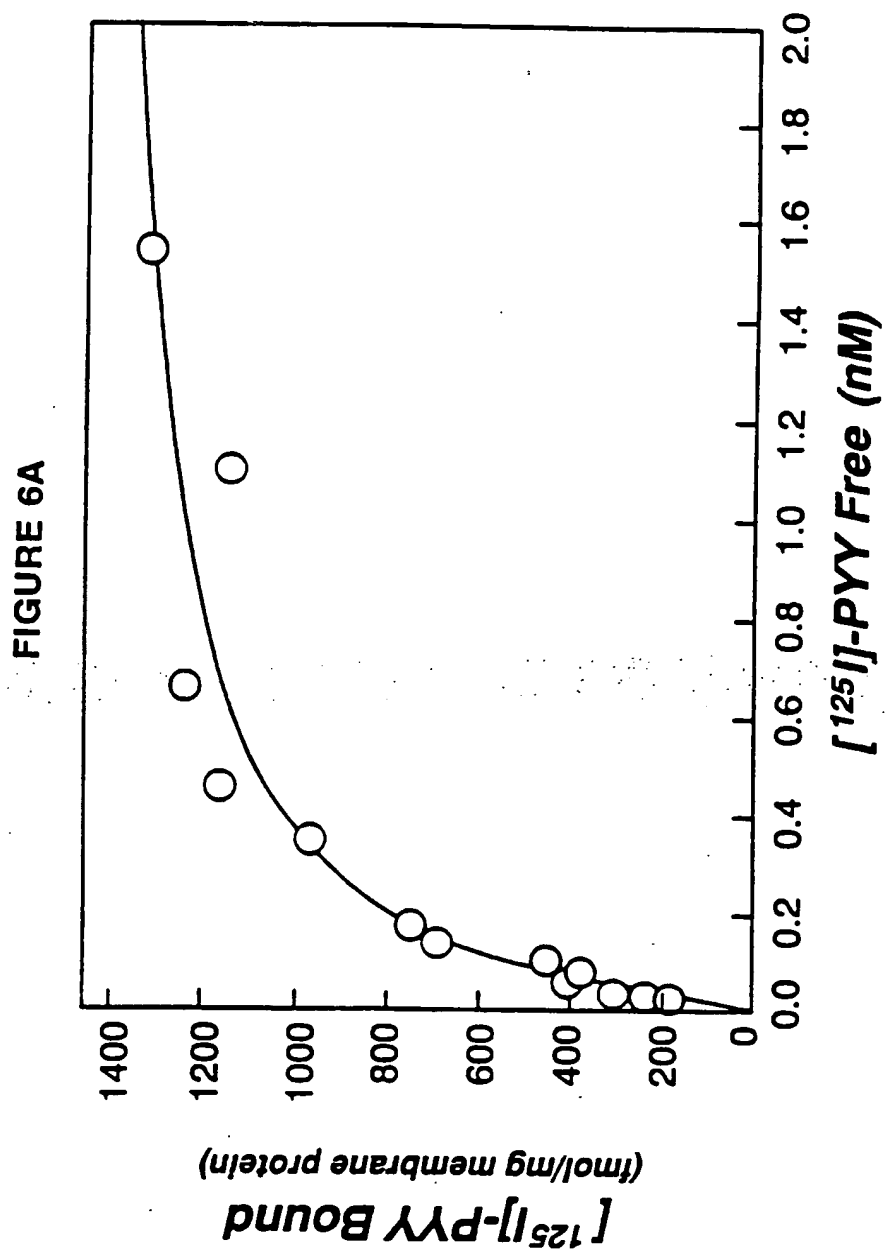


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FIGURE 5

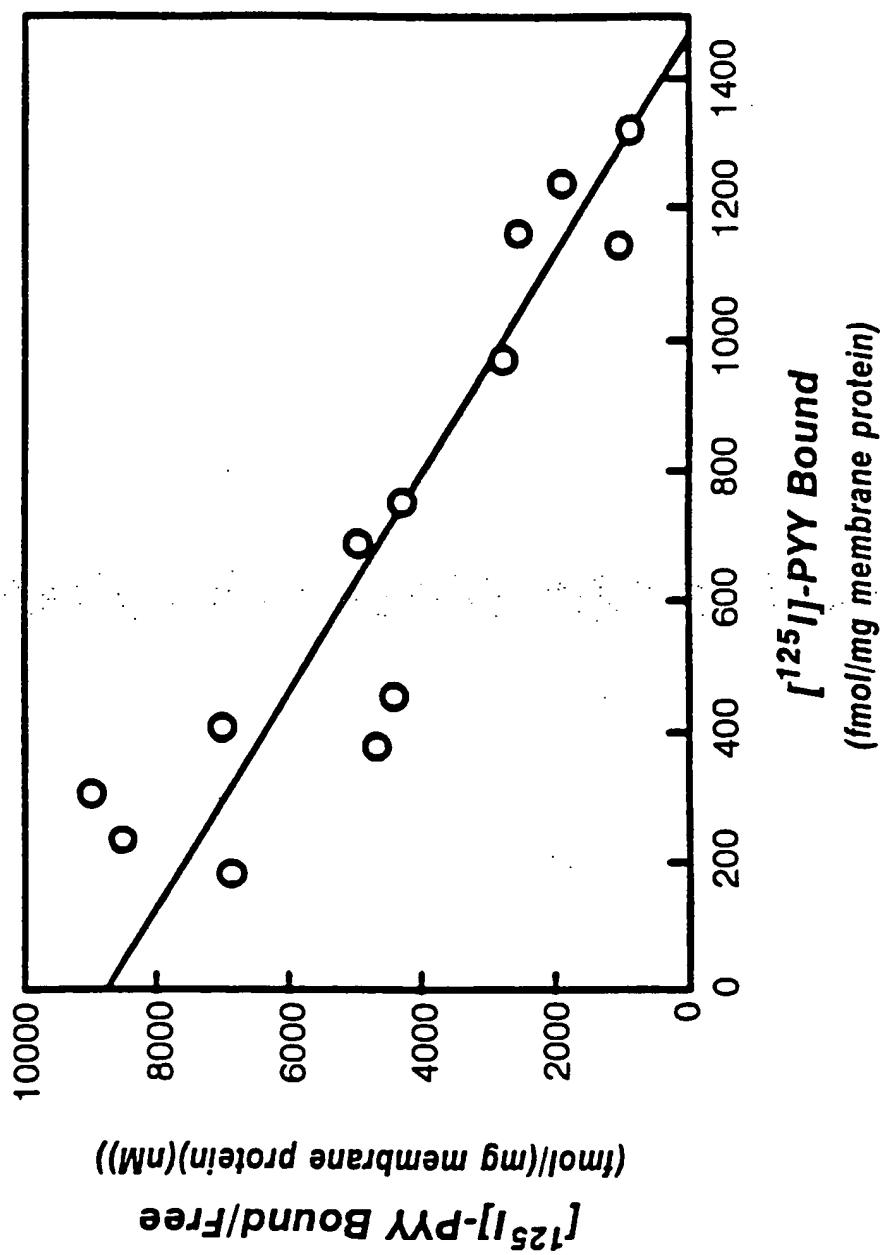


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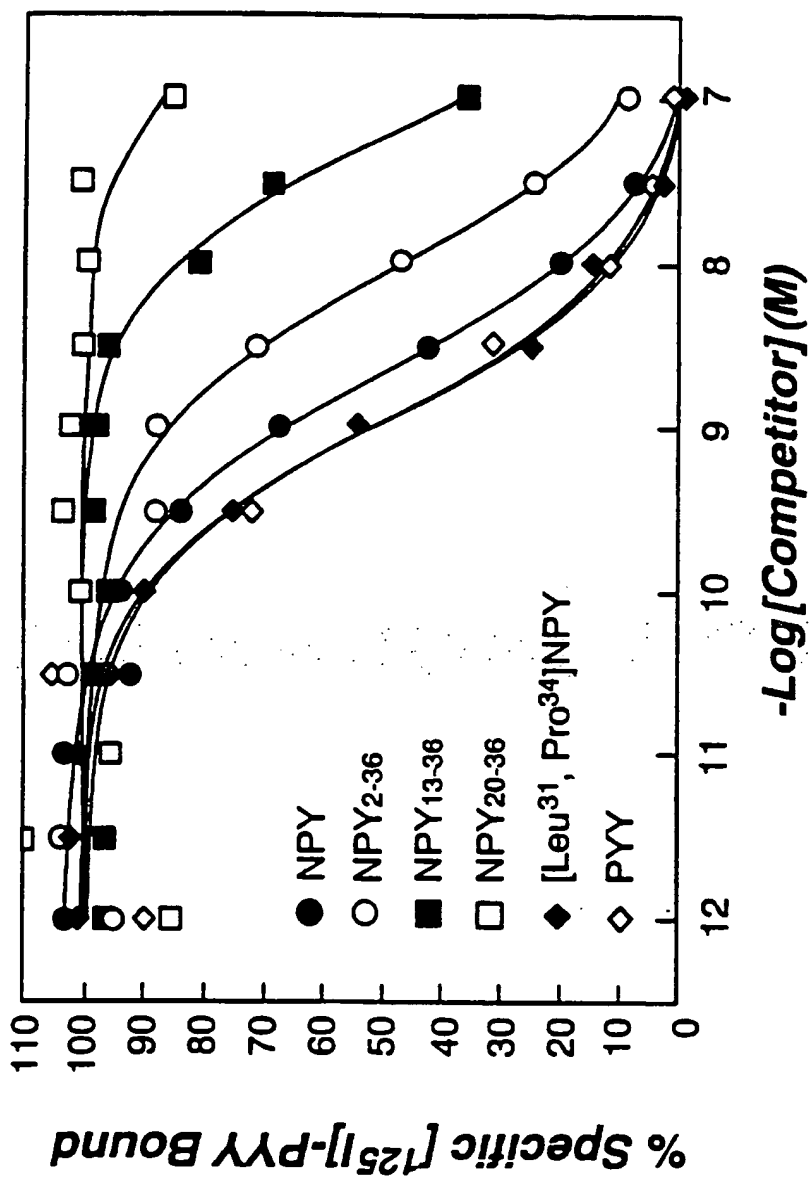
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FIGURE 6B



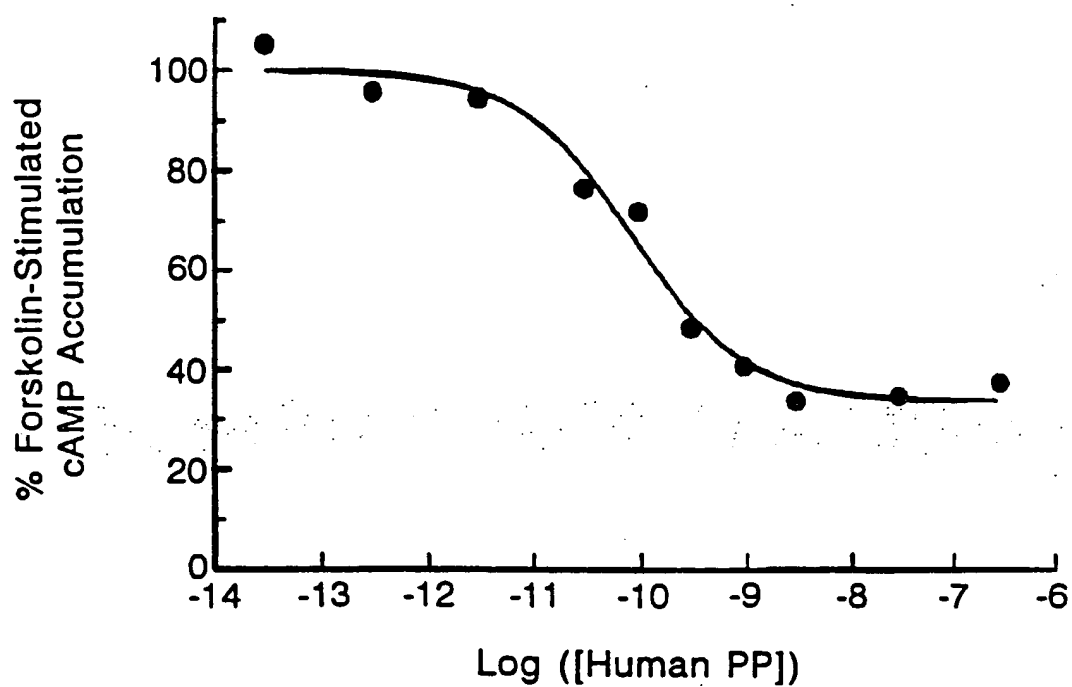
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FIGURE 7

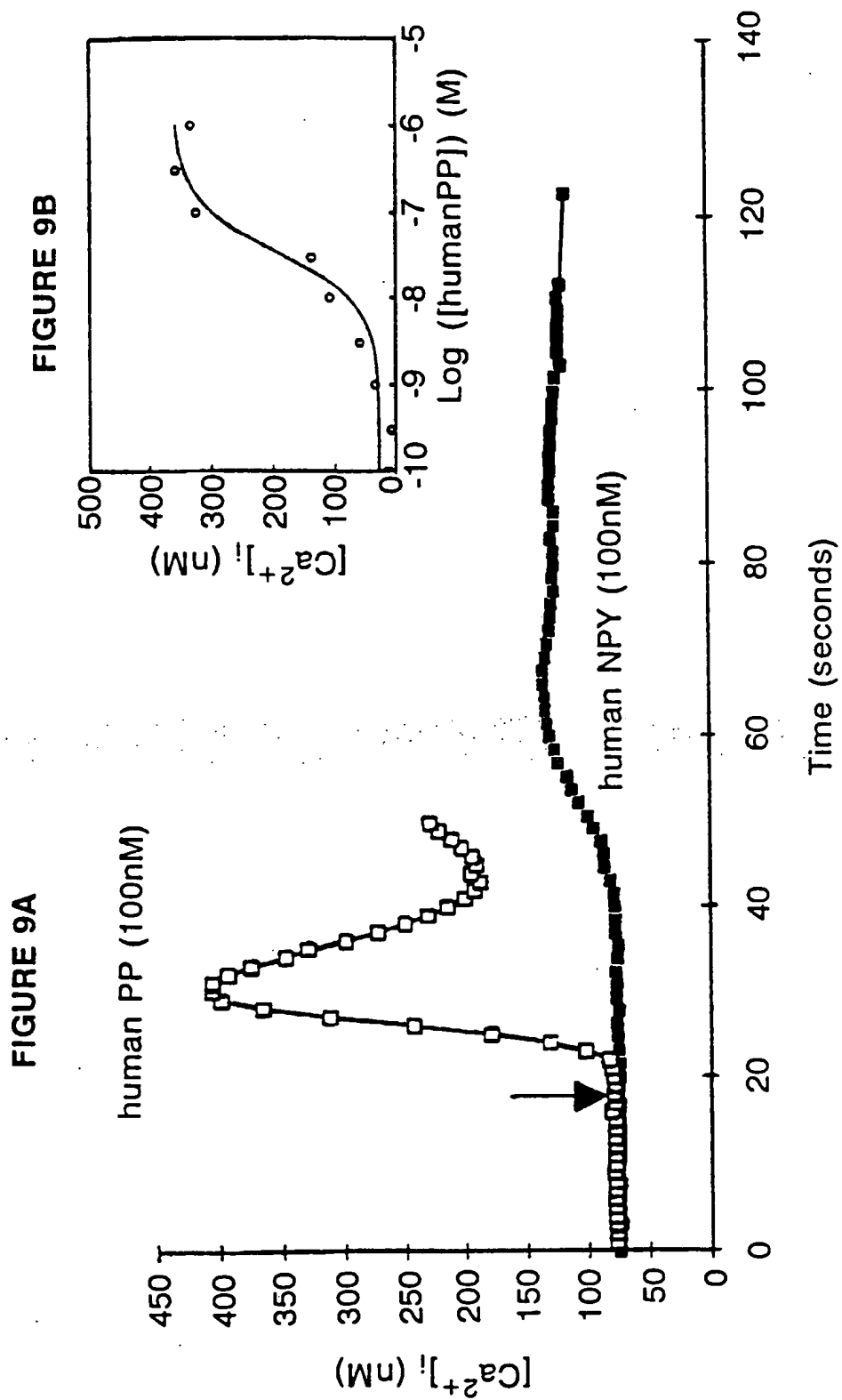


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FIGURE 8



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## INTERNATIONAL SEARCH REPORT

International application No.

PCT/US94/14436

<b>A. CLASSIFICATION OF SUBJECT MATTER</b> IPC(6) : Please See Extra Sheet. US CL : Please See Extra Sheet. According to International Patent Classification (IPC) or to both national classification and IPC		
<b>B. FIELDS SEARCHED</b> Minimum documentation searched (classification system followed by classification symbols) U.S. : 435/6, 7.1, 69.1, 240.1, 252.3, 252.33, 255.1, 320.1; 514/12; 530/350, 388.1; 536/23.1; 800/2 Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched Electronic data base consulted during the international search (name of data base and, where practicable, search terms used) APS, Biosis, Medline, WPI Search Terms: NeuropeptideY, Peptide YY, Pancreatic Peptide, receptor, cloning, DNA, antibodies, treatment.		
<b>C. DOCUMENTS CONSIDERED TO BE RELEVANT</b>		
Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
A	Annals of the New York Academy of Sciences, Volume 611, issued 15 November 1990, Wahlestedt et al, "Neuropeptide Y Receptor Subtypes, Y1 and Y2", pages 7-26, see pages 19-23.	1-146
A	US, A, 4,839,343 (WAEBER ET AL) 13 June 1989, see entire document.	117-136
A	Life Sciences, Volume 40, issued 1991, Wahlstedt et al, "Identification of Cultured Cells Selectively Expressing Y1-, Y2-, or Y3-Type Receptors for Neuropeptide Y/Peptide YY", pages PL-7-PL-12, see entire document.	1-146
<input checked="" type="checkbox"/> Further documents are listed in the continuation of Box C. <input type="checkbox"/> See patent family annex.		
* Special categories of cited documents: *A* document defining the general state of the art which is not considered to be of particular relevance *E* earlier document published on or after the international filing date *L* document which may throw doubts on priority claim(s) or which is cited to establish the publication date of another citation or other special reason (as specified) *O* document referring to an oral disclosure, use, exhibition or other means *P* document published prior to the international filing date but later than the priority date claimed *T* later document published after the international filing date or priority date and not in conflict with the application but cited to understand the principle or theory underlying the invention *X* document of particular relevance; the claimed invention cannot be considered novel or cannot be considered to involve an inventive step when the document is taken alone *Y* document of particular relevance; the claimed invention cannot be considered to involve an inventive step when the document is combined with one or more other such documents, such combination being obvious to a person skilled in the art *Z* document member of the same patent family		
Date of the actual completion of the international search 20 MARCH 1995		Date of mailing of the international search report <b>11 APR 1995</b>
Name and mailing address of the ISA/US Commissioner of Patents and Trademarks Box PCT Washington, D.C. 20231 Facsimile No. (703) 305-3230		Authorized officer Sally P. Teng <i>W. K. 732</i> Telephone No. (703) 308-0196

## INTERNATIONAL SEARCH REPORT

International application No.  
PCT/US94/14436

## C (Continuation). DOCUMENTS CONSIDERED TO BE RELEVANT

Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
A	The Journal of Neuroscience, Volume 4, Number 9, issued September 1984, Lundberg et al, "Comparative Immunohistochemical and Biochemical Analysis of Pancreatic Polypeptide-Like Peptides with Special Reference to Presence of Neuropeptide Y in Central and Peripheral Neurons", pages 2376-2386, see pages 2378-2381.	1-146
A	The Journal of Biological Chemistry, Volume 267, Number 16, issued 05 June 1992, Larhammar et al, "Cloning and Functional Expression of a Human Neuropeptide Y/Peptide YY Receptor of the Y1 Type", pages 10935-10938, see pages 10936-10938.	1-146
A	Proceedings of the National Academy of Sciences, Volume 89, Number 13, issued 01 July 1992, Herzog et al, "Cloned Human Neuropeptide Y Receptor Couples to Two Different Second Messenger Systems", pages 5794-5798, see pages 5795-5797.	1-146



# INTERNATIONAL SEARCH REPORT

International application No.  
PCT/US94/14436

## Box I Observations where certain claims were found unsearchable (Continuation of item 1 of first sheet)

This international report has not been established in respect of certain claims under Article 17(2)(a) for the following reasons:

1. ☐ Claims Nos.:  
because they relate to subject matter not required to be searched by this Authority, namely:
  
2. ☐ Claims Nos.:  
because they relate to parts of the international application that do not comply with the prescribed requirements to such an extent that no meaningful international search can be carried out, specifically:
  
3. ☐ Claims Nos.:  
because they are dependent claims and are not drafted in accordance with the second and third sentences of Rule 6.4(a).

## Box II Observations where unity of invention is lacking (Continuation of item 2 of first sheet)

This International Searching Authority found multiple inventions in this international application, as follows:

Please See Extra Sheet.

1. ☐ As all required additional search fees were timely paid by the applicant, this international search report covers all searchable claims.
2. ☒ As all searchable claims could be searched without effort justifying an additional fee, this Authority did not invite payment of any additional fee.
3. ☐ As only some of the required additional search fees were timely paid by the applicant, this international search report covers only those claims for which fees were paid, specifically claims Nos.:
  
4. ☐ No required additional search fees were timely paid by the applicant. Consequently, this international search report is restricted to the invention first mentioned in the claims; it is covered by claims Nos.:

Remark on Protest

- ☐ The additional search fees were accompanied by the applicant's protest.  
☐ No protest accompanied the payment of additional search fees.

# INTERNATIONAL SEARCH REPORT

International application No.  
PCT/US94/14436

## A. CLASSIFICATION OF SUBJECT MATTER:

IPC (6):

A61K 38/17; C07K 14/705, 16/00; C12N 15/09, 15/63, 15/70, 15/81; C12Q 1/00

## A. CLASSIFICATION OF SUBJECT MATTER:

US CL :

435/6, 7.1, 69.1, 240.1, 252.3, 252.33, 255.1, 320.1; 514/12; 530/350, 388.1; 536/23.1; 800/2

## BOX II. OBSERVATIONS WHERE UNITY OF INVENTION WAS LACKING

This ISA found multiple inventions as follows:

This application contains the following inventions or groups of inventions which are not so linked as to form a single inventive concept under PCT Rule 13.1. In order for all inventions to be examined, the appropriate additional examination fees must be paid.

Group I, claims 1-42, 49-54, 61-70, 72-87, 94-113, and 146, drawn to the Y4 receptor and its encoding nucleic acid, classified in Class 435, subclass 69.1.

Group II, claims 43-48 and 133, drawn to the Y4 receptor antibody and an assay for using the antibody, classified in Class 530, subclass 388.22.

Group III, claims 55-60, 134-136, and 140, drawn to a transgenic mammal, classified in Class 800, subclass 2.

Group IV, claims 71, 88-93, 114, 115, 117-132, 137-139, and 141-143, drawn to ligands that interact with the receptor and methods of treatment using the ligands, classified in Class 514, subclass 1.

Group V, claims 116, 144, and 145, drawn to a method of diagnosing a predisposition, classified in Class 435, subclass 6.

The inventions listed as Groups I-V do not relate to a single inventive concept under PCT Rule 13.1 because, under PCT Rule 13.2, they lack the same or corresponding special technical features for the following reasons: Groups I, II, III, IV, and V are directed to materially distinct products and materially distinct methods of using the products. The inventions are not required for each other because the products are structurally and functionally different, and the methods have different goals, steps, and reagents. Additionally, Groups I and V include different methods of using the probes of Group I. The claims are not so linked by a special technical feature within the meaning of PCT Rule 13.2 so as to form a single inventive concept.